Synthesis and in vitro evaluation of bioreductively activated prodrugs of anti-inflammatory agents

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Synthesis and *in vitro* Evaluation of Bioreductively Activated Prodrugs of Anti-inflammatory Agents.

submitted by

Sandra Ferrer

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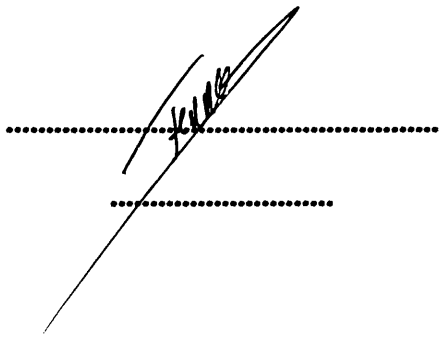
of the University of Bath

2001

The research work in this thesis has been carried out in the Department of Medical Sciences, under the supervision of Dr Michael D. Threadgill, Dr Declan P. Naughton and Prof. David R. Blake.



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ABSTRACT

Rheumatoid arthritis is a widely spread disease of which causes remain relatively unexplained. The existing therapies for RA have shown limited effects; one reason for that being the presence of hypoxia in the rheumatic joint. This decreased level of oxygen in the tissues is the consequence of the inadequate perfusion of the joint due to defficient distribution and quality of the vasculature resulting from angiogenetic processes and pressure rises triggered by increased inflammation and exercise, occluding parts of the capillary bed. Hypoxia is responsible for important physiological changes which render conventional treatments inactive, and triggers the production of entities such as reactive oxygen species and poly(ADP-ribose)polymerase.

The aim of the present study is to use a bioreductive compound or carrier for the specific targeting of a drug to areas of hypoxic and/or ischaemic tissues, in which the desired drug species is linked to a non-cytotoxic counterpart.

Two series of novel potentially bioreductivatable prodrugs bearing anti-inflammatory, anti-arthritic agents have been synthesised for the selective delivery of these drugs to areas of inflammation characterised by low oxygen concentrations.

The first series is based on a 1,2-dimethyl-5-methoxyindole-4,7-dione trigger. The synthesis of the first potential prodrug 1-[(1,2-dimethyl-4,7-dioxo-5-methoxyindol-3-yl)methyl]-4-(prednisolone-21-yl) butanedioate resulted from the successful nucleophilic attack from the steroidal drug moiety on the trigger. The PARP inhibitors isoquinolin-1-one, and its 5-bromo and 5-iodo-analogues were used for the synthesis of four prodrugs. The Mitsunobu reaction was used to link the trigger and effector. The site of attachment of the two subunits varied with the drug moity used. The non-substituted isoquinolin-1-one gave the ester derivative whereas N-alkylation took place in the case of the 5-bromoisoquinolinone. 5-Iodoisoquinolin-1-one gave both derivatives separately.

The second series is 5-nitrothiophene-2-ylmethyl-based. The nucleophilic species generated from 5-nitro-2-thienylmethyl derivatives reacted with acetylsalicyloyl chloride to give the aspirin prodrug 5-nitrothien-2-ylmethyl 2-acetoxybenzoate. Coupling of a nitrothienylmethyl succinate active ester with prednisolone generated the steroid prodrug 1-[(5-nitro-2-thienyl)methyl]-4-(prednisolone-21-yl) butanedioate. The Mitsunobu reaction between 5-nitro-2-thienylmethanol and isoquinolin-1-one and its 5-iodo and 5-bromo analogues gave the ether derivatives. No N-alkylation was observed.

The release studies were carried out using chemical systems representing *in vivo* reductive conditions. Drug release was monitored by HPLC and, in a new development, by NMR. Release was observed for the indole-isoquinoline ethers after stoichiometric reduction of the potential prodrugs but the N-alkylated and the steroid derivatives gave no signs of drug release.

Reduction of the nitrothiophene trigger was observed for each target, and release of the isoquinolinone effector moiety was detected. The aspirin and steroid prodrugs seemed to undergo hydrolysis of the linking ester bonds before reductive trigger activation.

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Finally, I would like to thank my family and friends, especially Maurizio Varrone without who the release study would not have been possible, but also for helping me in so many ways.

This thesis is dedicated to *Ines Leon Ferrer* and *Angel Ferrer.*

"To Her, as I know she is watching over me"

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ABBREVIATIONS

ADEPT	antibodydirected enzyme-prodrug therapy
ADP-ribose	adenosine diphosphoribose
Ag	antigen
5-AIQ	5-aminoisoquinolin-1-one
APC	antigen presenting cell
Aq.	aqueous
ATP	adenosine trisphosphate
AZQ	diaziquone
Boc	*tert*-butoxycarbonyl
bp	boiling point
Bu^t	*tert*-butyl
CNS	central nervous system
CG	cytosine guanine
Conc.	concentrated
COX 1/2	cyclooxygenase 1/2
d	days
Da	Daltons
DAF	delay accelerating factor
DBD	deoxyribonucleic acid binding domain
DBU	1,8-diazabicyclo[5,4,0]-undec-7-ene
DCM	dichloromethane
DEAD	diethyl azodicarboxylate
DIBAL	diisobutylaluminium hydride
DMARD	disease-modifying anti-rheumatic drug
DME	1,2-dimethoxyethane
DMF	N,N-dimethylformamide
DMFDMA	N,N-dimethylformamide dimethylacetal
DNA	deoxyribonucleic acid
E_7^1	one-electron reduction potential in aqueous solution at pH 7.0
EI	electron impact

FAB	fast atom bombardment
GDEPT	gene-directed enzyme-prodrug-therapy
GMC-SF	granulocyte macrophage colony stimulating factor
GR	glucocorticoid receptor
h	hour
HCR	hypoxic cytotoxicity ratio
HGF	hepatocyte growth factor
HIF	hypoxia inducible factor
HPLC	high perfomance liquid chromatogrphy
HRE	hormone response element
HSC	hypoxia-selective cytotoxin
IC_{50}	concentration causing 50% inhibiton
IFN	interferon
IgG	immunoglobulin G
IL	interleukin
iNOS	inducible nitric oxide synthase
IR	infrared
LDB	ligand binding domain
Me	methyl
MCP	monocyte chemoattractant protein
MHC	major histocompatibility complex
MIP	macrophage inflammatory protein
MMC	mitomycin C
mp	melting point
MS	mass spectroscopy
m/z	mass to charge ratio (mass spectroscopy)
NAD	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate (reduced form)
Nk	natural killer cell
NMR	nuclear magnetic resonance
NOS	nitric oxide synthetase
NSAID	non-steroidal anti-inflammatory drug
NTR	nitroreductase
PARP	poly(adenosine diphosphoribose)polymerase

PMM	pentamethylmelamine
Pr^i	isopropyl
RA	rheumatoid arthritis
RF	rheumatoid factor
RNA	ribonucleic acid
ROS	reactive oxygen species
Rt	retention time
SAARD	slow-acting anti-rheumatic drug
S_N	nucleophilic substitution
TBAF	*tetra*-butylammonium fluoride
TBAB	*tetra*-butylammonium bromide
TCR	T cell receptor
TFA	trifluoroacetic acid
TGF	tumour growth factor
Th	lymphocyte T helper cell
THF	tetrahydrofuran
TLC	thin layer chromatography
TNF	tumour necrosis factor
UV	ultraviolet
VCAM	vascular cell adhesion molecule
VEGF	vascular endothelial growth factor
XDH	xanthine dehydrogenase
XO	xanthine oxidase

INTRODUCTION

1. RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is one of the many forms of musculoskeletal disorders. It is an inflammatory arthropathy, one of the six main classes of musculoskeletal disorders. These can be further subdivided to include the main categories of rheumatic disorders.[1,2,3]

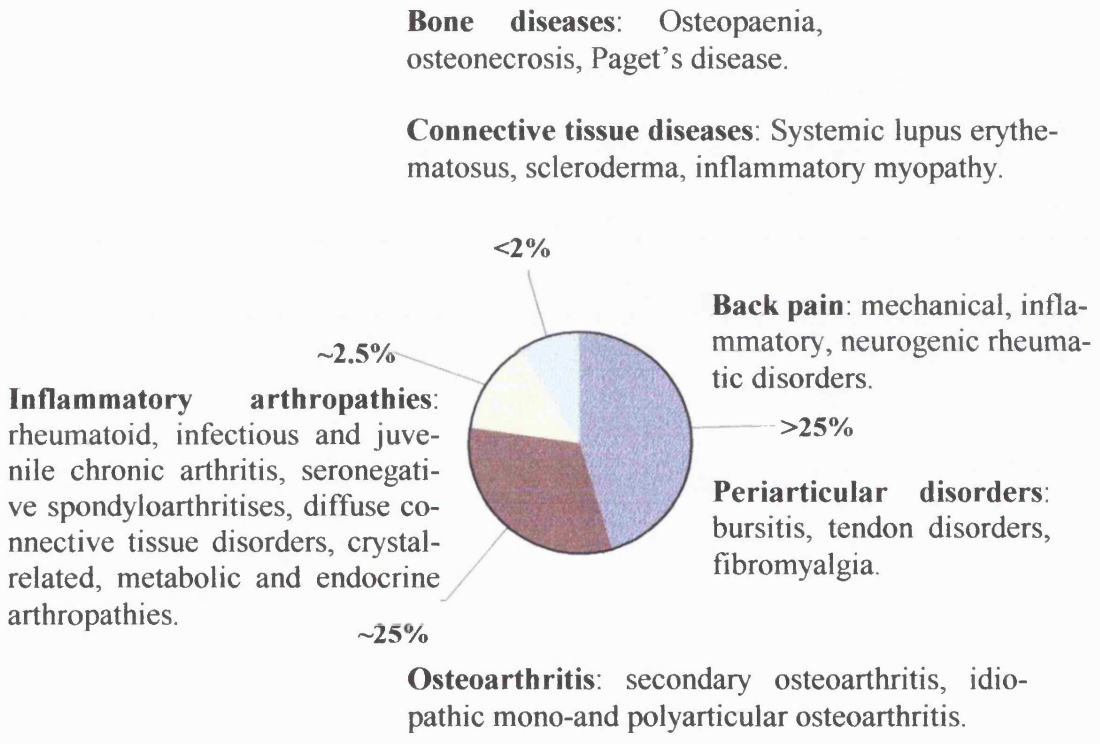


Figure 1: Musculoskeletal disorders and their distribution in the population concerned.

Although back pain and periarticular disorders seem to be the most common (> 25%), followed by osteoporosis and osteoarthritis (20 to 30%), inflammatory arthropathies still represent around 2.5% of the population presenting musculoskeletal disorders and are often more severe (**Figure 1**).[1,2,3]

RA has a worldwide distribution and affects 1 to 3% of the population (1% in the UK). The disease often begins in middle age and increasingly occurs in older people, but children and young adults are also touched. Typically the most common age of onset is 30-50. Women before the menopause are affected 2 to 3 times more then men. After the menopause, the frequency of onset is similar between sexes.[4,5]

RA, like all other types of arthritis, has a considerable financial and social impact. The main impact being through the very high rates of disabilities but the rate of mortality is very low (0.02%). Other major effects of the disease, such as pain but also depression, anxiety and feelings of helplessness impair the abilities of people severely touched by RA to carry out normal daily activities. Furthermore the medical and surgical treatments together with the loss of income add up to considerable costs.[4,5]

1.1 *THE MAIN FEATURES OF RHEUMATOID ARTHRITIS*

RA is a chronic symmetrical polyarthritis. It is a systemic disorder characterised by chronic inflammatory synovitis (inflammation of the synovial tissue) of mainly peripheral joints. Its course is extremely variable and is associated with non-articular features. It causes pain, swelling, stiffness and loss of functions in the joints. In the main, peripheral joints are affected, such as fingers and wrists, but it has been shown that the disease involves the tendons, tendon sheaths and bursae in some patients. Other organs, e.g. skin, eyes, peripheral nerves, pleura, pericardium and the heart itself could also be touched.[3,5]

RA often occurs symmetrically. If one hand is involved, the other will be.
The disease varies from one person to another; it can last a few months or years and disappear without causing noticeable damage. It can be moderate with periods of flares and remissions. It can also be severe, active at most times, last for years and lead to joint damage and disability.[3,5]

1.2 *PATHOLOGY.*

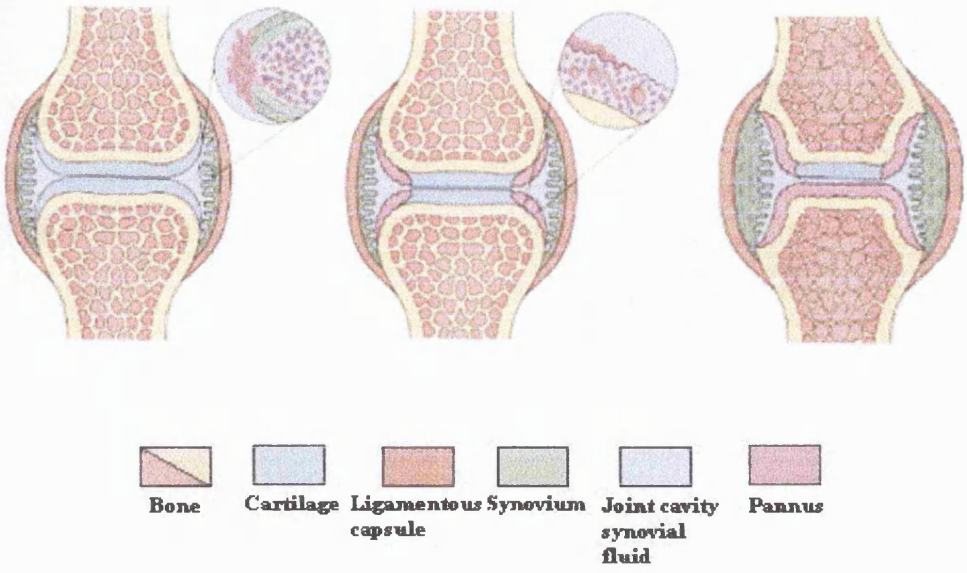


Figure 2: Schematic view of the effects of RA on a human knee joint. [3]

A normal joint is surrounded by a ligamentous capsule that protects it and offers resistance to pressure in any direction. The joint capsule is lined with a type of tissue called synovium. This produces synovial fluid, which is responsible for lubricating and nourishing the cartilage and bones inside the joint capsule. [1,2,3]

When RA occurs, the immune system attacks the cells of the joint capsule. The reasons for this are still unclear. The production of rheumatoid factors (RFs) by plasma cells in the synovium and the local formation of immune complexes are thought to play an important part leading to rheumatoid synovitis (inflammation of synovial lining of joints, tendon sheaths and bursae). [1,2,3]

The synovium becomes greatly thickened and results in a joint that is swollen and puffy to the touch. The synovium proliferates into a villous pattern and is infiltrated by a variety of inflammatory cells, including polymorphs, which migrate through the tissue into the joint fluid, lymphocytes and plasma cells. The normally thin surface layer of lining cells becomes hyperplastic and thick. There is severe angiogenesis and increased permeability of blood vessels which, in the synovial lining layer, leads to joint effusions containing lymphocytes and dying polymorphs. Activated lymphocytes

and macrophages in the synovium produce a rich mixture of cytokines, including interleukins, prostaglandin and tumour necrosis factor alpha (TNFα).[1,2,3]

The growing synovium spreads from the joint periphery onto the cartilage surface. This inflamed spreading pannus blocks the normal route for cartilage nutrition and, by the conjugated effect of cytokines on the chondrocytes, the cartilage gets damaged. It becomes thinner, exposing the underlying bone. During active synovitis, local cytokine production and the lack of joint mobility combine to cause juxta-articular osteoporosis (**Figure 2**).

The proliferating synovium also grows between the synovial margins and the epiphyseal bone cavity and damages the bone, responsible for "erosions" seen on X-rays. Those are generally irreversible and are associated with joint deformity.[1,2,3]

1.3 *IMMUNOLOGY.*

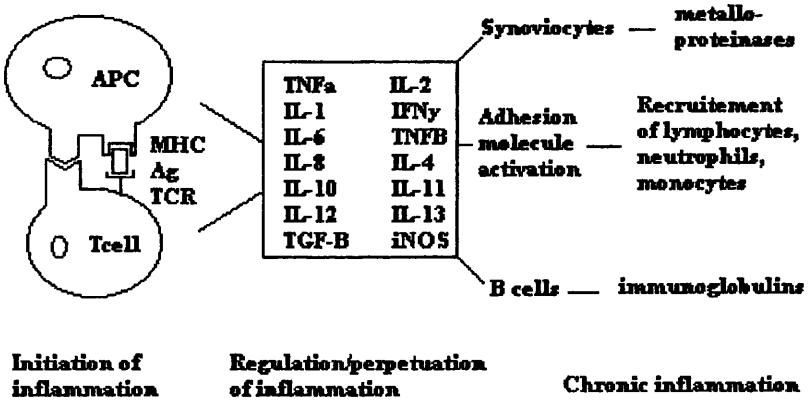


Figure 3: Inflammatory cells involved in rheumatoid inflammation (APC: antigen presenting cell, Ag: antigen, MHC: major histocompatibility complex, TCR: T cell receptor, iNOS: inducible nitric oxide synthase, TNF: tumour necrosis factor, IL: interleukins).[6]

The chronic synovial inflammation may be caused by the continuous T-cell activation or may be maintained by the local production of rheumatoid factors and persistent

stimulation of macrophages *via* IgG Fc preceptors[3,4,5]. However, it was observed that minimum amounts of the inflammatory factors are produced by T-cells (interferon and interleukins IL-2 and IL-4). The mechanism of T-cell activation might, therefore, not be the target of choice. On the other hand, the cytokines (IL-1, IL-8, TNFα, granulocyte macrophage colony stimulating factor, GMC-SF) and chemoattractive cytokines produced by macrophages (macrophage inflammatory protein MIP, and monocyte chemoattractant protein MCP) and fibroblasts (IL-6) are abundant. Tumour necrosis factor alpha (TNFα) is expressed in significant quantities; it is known to play a pivotal role in the disease and is an interesting target for therapy [6,7](**Figure 3**).

Synovial fibroblasts have high levels of the adhesion molecules, such as vascular cell adhesion molecules (VCAM-1), which support B lymphocyte survival and differentiation. They may facilitate the formation of abnormal lymphoid tissue in the synovium[6,7]. The triggering antigen remains unclear. The hypothesis has been made that immunoglobulins could become antigenic in RA following deficient glycolysation[2,4]. There is little evidence that collagen type II is the triggering antigen, although it is a cause of arthritis in animal models. Bacterial or slow virus infections have been implicated but they are unproven as causes.[2,4]

1.3.1 *Rheumatoid factors*

Rheumatoid factors (RFs) are circulating antibodies (autoantibodies), directed against altered or negative gammaglobulin. They occur as IgM, IgG and IgA immuno-globulins and have specificity for antigenic determinants on the Fc fragment. RFs are usually a complex of an IgM acting as an antigen with another acting as the antibody[2,4]. The temporary production of RFs is an essential part of the body's normal mechanism for removing immune complexes but, in RA, their production is persistent and occurs into the joint[2,4]. They may be of any immunoglobulin class (IgM, IgG, or IgA), but the most common tests employed clinically detect IgM rheumatoid factor. Around 70% of patients with polyarticular RA have serum IgM rheumatoid factor in the serum. When the standard test for IgM is persistently negative, the term seronegative RA is used. Seronegative patients tend to have a less severe case of

synovitis. RFs are not found in synovitis associated with psoriasis, ankylosing spondylitis and inflammatory bowel disease, or in reactive arthritis. IgM rheumatoid factor cannot be used as a diagnostic for RA; it can only be extra information to predict the severity of the disease. A persistently high titre in early disease implies more persistently active synovitis, more joint damage and greater disability eventually.[2,4]

1.4 *THE TREATMENTS.*

There are a variety of approaches to treat RA. The objectives of the treatment are to:

- Relieve pain
- Reduce inflammation
- Slow down or stop joint damage
- Improve the patient's sense of well-being and ability to function

Patients with RA may have to adapt their lifestyle to the disease, find a balance between rest and exercise, take care of the joints involved (use of splint), reduce their level of stress and go on a healthful diet.

Surgery is still one of the major answers for patients with severe conditions; this involves joint replacement, tendon reconstruction, and synovectomy (hardly used nowadays) but, above all, medication is the most common therapy for RA (**Table 1**).[5,8,9]

Medication	Uses/ effects	Side effects
NSAID *nonsteroidal anti-inflammatory drugs* plain and buffered aspirin, *ibuprofen*, ketoprofen, naproxen, diclofenac, diflunisal	Reduction of pain swelling and inflammation. Generally part of early and continuing therapy.	Stomach disorder tendency to bruise easily, fluid retention ulcers possible kidney and liver damage.
DMARDs *Disease-modifying anti-rheumatic drugs* or ***SAARDs*** *slow-acting antirheumatic drugs* Gold (myochrisine, ridaura) Antimalarials (hydroxychloroquine) Penicillamine sulfasalazine	Alteration of the course of the disease and prevention of joint and cartilage destruction. May produce significant improvement for many patients Mechanism of action unknown. Patients can use several over the course of the disease Takes a few weeks or months to have an effect.	**Toxicity** Gold: skin rash, mouth sores, stomach and kidney disorders, low-blood count. Antimalarials: stomach and eye disorders Penicillamine: skin rash, stomach and kidney disorders, blood abnormalities. Sulfasalazine: stomach disorders
Immunosuppressants (*DMARDS*) Methotrexate Azathioprine Cyclophosphamide	Restriction of the overly active immune system Potential toxicity diminishing effectiveness over time Methotrexate can result in rapid improvements, seems effective. Azathioprine used for patients not responding to other drugs Chemotherapeutic agent. Used in combination therapy Cyclophosphamide chemotherapeutic agent, used only in very severe cases of RA for potential toxicity.	**Toxicity** Methotrexate: stomach and liver disorder, low white blood cell count Azathioprine : potential blood abnormalities, low white blood cell count, increased cancer risks. Cyclophosphamide : low white blood cell count, other blood abnormalities, increased risk of cancer.
Corticosteroids (glucocorticoids) Prednisone, methylprednisolone	anti-inflammatory and immuno-suppressive effects Dramatic improvement in very short time Used early while waiting for DMARDS to work Used for severe flares and when no response to NSAIDs and DMARDs	Osteoporosis, mood changes, fragile skin, easy bruising, fluid retention, weight gain, muscle weakness, onset or worsening of diabetes, cataracts increased risk of infection, hypertension, potential for serious side effects at high doses

Table 1: Four classes of treatments used for RA, drugs, effects and side effects.[5,8,9]

1.5 *INFLAMMATION, ANGIOGENESIS AND HYPOXIA.*

Although we have seen that the immunological reasons behind RA are still unclear, research has allowed the slow dissection of some important mechanism and phenomena. Those are the elements permitting the development of therapies.
Different groups have reached different conclusions on what is the main driving force in RA, setting different priorities for the target to reach in the development of new therapies.

1.5.1 *Angiogenesis*

Angiogenesis is the process by which new blood vessels are created. This constitutes one of the earliest changes within the rheumatic joint; blood vessels develop providing nutrients, oxygen and cells to the growing pannus[2,4]. During pathological angiogenesis, endothelial cell activation, *via* various cytokine mediators and/or adhesion molecules, is abnormally prolonged as a result of an imbalance between angiogenesis-enhancing and inhibiting factors[2,4].

By highlighting the similarities between synoviocytes and tumour cells, Weber *et al*[10] propose that angiogenesis promotes rheumatoid synovitis in a similar way that it promotes tumour progression. The hypothesis relies on the close relation between inflammation and angiogenesis. Vascular cell adhesion molecule (VCAM–1) and E-selectine adhere to endothelial cell surface; they are cleaved by lymphocytes and released as soluble molecules. They induce a chemoattractant effect on neighbouring vascular cells thus causing early activation of angiogenesis.

Angiogenic factors are thought to play an important part in the following stages, namely interleukin 8 (IL-8), tumour necrosis factor alpha (TNFα), vascular endothelial growth factor (VEGF), and hepatocyte growth factor (HGF) released by macrophages and lining layer cells. [10]

1.5.2 *Hypoxia and rheumatoid arthritis*

The synovial intra-articular pressure observed in a normal joint is subatmospheric (between 2 and 10 mm Hg), whether the joint is in motion or at rest. The maintenance of this pressure ensures that the blood vessels remain patent on exercise. In the rheumatic joint, the inflammation induces pressure rises, which increase further during exercise, exceeding the capillary perfusion pressure; parts of the capillary bed are consequently occluded, inducing hypoxia. The synovium is described as chronically hypoxic in RA[7,11,12]. Hypoxia is a major stimulant of VEGF, which is thought to play an important role in the regulation of angiogenesis *via* the production of hypoxia inducible factor (HIF-1).[10,13]

Chronic rheumatoid synovitis might be a consequence of persistent angiogenesis.[13] The normal synovial membrane is densely vascularised and is composed of one or two layers of synovial cells. In RA, the synovial layer becomes hyperplastic and locally invasive. Fibroblast proliferation and important neovascularisation occur. Inflammatory infiltrates are present. The new capillaries are generally redistributed towards the deeper parts of the synovium and the vascular density remains hardly changed. The new blood vessels contain a high level of pericytes, and a low level of angiotensin-converting enzyme, overexpression of E-selectine, integrin $\alpha v\beta 3$ and metalloproteinase. The endothelial cells express markers for proliferation (similar to tumour and skin repair proliferation). The simultaneous proliferation and apoptosis of endothelial cells explain the *quasi* non-existent change in vascular density. The accelerated vascular turnover may result in deficiencies in the new vascular system like absence of vasoregulating neuropeptide receptors (substance P), and lack of functionality. Those deficiencies prevent tissue healing and induce persistent hypoxia and acidosis, which trigger more inflammation, pain and tissue damage inducing more severe arthritis.[13]

Another approach was developed by Blake [6,7] and Bodamyali *et al* [7], who propose that hypoxia is the main driving force in RA.

T_{helper} lymphocytes have been shown to play part in RA. T_{h1} response is characterised by TGFβ, IFNγ, TNFα, IL-1 and IL-2 expression. T_{h2} seems to show increased

production of IL-4, IL-5, IL-10, and IL-13 (anti-inflammatory cytokines). Levels of TNFα and IL-1 are high in RA whereas the level of IL-2 is low which constitutes an abnormality in the T_{h1} response profile[6,7].

Previous studies[7] have shown that hypoxia up-regulates TNFα and IL-1 and down-regulates IL-2. The predominant expression of a certain type of cytokines may influence T_{h1} / T_{h2} differentiation. Furthermore, this differentiation takes place after binding of the cytokines to the T cell precursor receptors and is mediated by phosphorylation of the relevant signal transducers and activators of transcription. Hypoxia appears to influence both cytokine production and phosphorylation and, therefore, would be responsible for the T_{h2} to T_{h1} switch.

Although T-cell cytokines are thought to be involved in the development of abnormal inflammatory response, studies have shown that patients with RA and HIV still suffer from destructive RA pathology when all immune activity has ceased (production of T-cells and cytokines has stopped). In RA, the apparent T cell hyporesponsiveness is also suggested to be related to hypoxia[7]. Under hypoxia, there is an increased production of ketones during glycolysis. These are available to form an increased number of Schiff bases with endogenous primary amines. The formation of these Schiff bases regulates the interaction of T cells and antigen-presenting cells. Thus the nature and concentration of carbonyl precursors for the carbonyl-amine condensation will influence the inhibition of T cell responses and may lead to T cell anergy. Finally, the growth factor and cytokine profiles of rheumatoid synovial cells *in vivo* match more closely to those of macrophages and fibroblasts *in vitro* than they do to those of T cells *in vitro*. This suggests that those cytokines and growth factors would be produced by the synovial cells rather than by T-lymphocytes. It is also known that hypoxia influences macrophage-derived expression of cytokines (IL-1, IL-6, TNFα, GM-CSF).[7]

All this evidence, according to Bodamyali *et al* [7], suggests that hypoxia, rather than angiogenesis, induces chronic RA. They also report that studies of the vascular system of rheumatic synovium brings some more evidence. The capillary density in the rheumatic synovium is one third of the one observed in normal synovium, the distance

between capillaries and joint cavity increases in RA, indicating inadequate perfusion. The pannus was shown to be relatively avascular and mainly hypoxic. Bodamyali *et al* [7] then conclude that there is obvious lack of angiogenesis and that hypoxia is the main factor causing chronic synovitis.

The rapid review of those two approaches suggests that the immune mechanism of RA is complex, with pleiotropic cytokines and redundancy in some of the regulatory networks. It may then be necessary to target different sites of the immune cascade at the same time.

Walsh[13] showed that targeting the identified important factors inducing angiogenesis sets the problem of selectivity, since vascular growth is a normal mechanism in humans observed in tissue repair and female reproduction.

Chikanza[6] drew attention to the fact that none of the existing treatments for RA can be considered to be curative and definitive therapies for the disease. Those reported are in development, and based on different strategies: T cell targeting, monocytes/ macrophages derived cytokines inhibition, adhesion molecule inhibition, antioxidant, free radical scavengers and NO production inhibition, bioreductive cytotoxic agents delivery, induction of immune tolerance (collagen therapy, subreum), inhibition of metalloproteinases, induction of apoptosis, hormonal immunomodulation.[6,14]

Although it is clear that targeting of cytokines and sites of the inflammatory cascade is an important mean of regulating RA, it is important to remember that cytokines are highly important in normal homeostasis. Selective blockade of disease-causing or potentiating cytokines could induce serious adverse effects.

One of the characteristics of the rheumatic joint that has been underlined several times so far is the presence of hypoxia[15,16]. This low oxygen concentration makes the tissues very different to oxic tissues from a physiological but also biological point of view and may be of importance for the specific targeting of the tissues touched by the disease.

2. HYPOXIA

Hypoxia was first described and identified in solid tumours[17]. Tumour cells proliferate more rapidly than their supporting vasculature. They also invade, compress, injure or obliterate blood vessels, leading to a permanently interrupted blood flow. Poor tumour vascularisation is thought to result in poor delivery of oxygen leading to the presence of chronically hypoxic tumour cells. A fraction of the hypoxic region is composed of transiently hypoxic cells, resulting from a temporary interruption of the blood flow that may last for seconds or minutes.[10]

Tumour venous and capillary vascular morphology and physiology are abnormal, as demonstrated by phenomena such as dilated and tortuous veins, sinusoids, arteriovenous shunting and vascular channels with incomplete vascular endothelium. Blood flow and red cell velocity are much lower than in normal tissues. The low blood flow is believed to be due to a vastly expanded venular and capillary network without a concomitant increase in arterial supply.[18]

In RA, the hypoxic nature was suggested originally on the basis of measurements of oxygen concentration within the inflamed cavity. The description of the physiological and pathological events in tumours matches closely that of RA[6,12]. Synovial cells proliferate at a high rate to create this pannus membrane; angiogenesis is also accelerated but cannot keep pace with the rate of synovial thickening. Analysis of the capillary density showed it to be one third of that in normal synovium; the new blood vessels are distributed more deeply. The loss of highly organised vascular structure causes relatively less uniform perfusion through the tissue. Subsequent poor blood flow (blood occupies 2.9% of the volume of a normal joint but only 1.2% of that of a rheumatoid joint) aggravates hypoxia by diminishing the oxygen gradient out of the vessels[20]. The oxygen gradient can be explained by the fact that cellular respiration depletes oxygen as it diffuses from blood vessels through packed layers of cells, with chronic hypoxia occurring at distances of 150-200 μm from capillaries, where almost all the oxygen has been used.[20,21] In normal tissues, the oxygen concentration varies from 3.1% to 8.7% (oxygen partial pressure of 24 to 66 mm Hg) whereas concentrations measured in hypoxic tissue range from 1.3% to 3.9% (10 to 30 mm

Hg) [12,20,21]. With sustained hypoxia, the capillaries become paralysed and lose their vasoactive responses. In addition to the hypoperfusion, mobility of the inflamed joint results in increased intra-articular pressure, exceeding the capillary perfusion pressure. Parts of the capillary bed are occluded, inducing acute ischaemia and hypoxia. The qualitative blood vessel abnormalities previously described also prevent tissue healing and induce persistent hypoxia[6,12,15,16].

Many different techniques are available for the measurement of oxygen in tissues to assess hypoxia[20]. These include vascular density measurements, oxygen electrodes (most widely used and reliable), fibre optic biosensors, spectroscopic methods, bioreductive chemical probes (*e.g.* 2-nitroimidazoles) and intrinsic markers (*e.g.* proteins and mRNA)[20]. Expression of several glycolytic and respiratory enzymes and their mRNA is oxygen-dependent. In differentiating human myoblasts, hypoxia induces accumulation of RNA transcripts for glycolytic enzymes, such as pyruvate kinase and reduced levels of transcripts for enzymes used in oxidative energy metabolism[20].

As well as having diminished oxygen concentrations, hypoxic cells develop physiological perturbations that alter their behaviour and characteristics[17-22]. Hypoxia can result in an accumulation of cells in G1, arrest cells at their cell cycle position or prolong cell cycle times. This reduction in cell division is one of the reasons why chemotherapy is relatively inefficient for treating hypoxic tissues. Energy metabolism will be perturbed and depletion of ATP levels is observed (anaerobic oxidation of glucose produces less ATP than the Krebs cycle and increased Ca^{2+} concentration). RNA and protein synthesis will be decreased but specific stress proteins may be induced and synthesised.[12,17,20,22] The concentration of cytolytic Ca^{2+} is increased, inducing decreased energy production, increased energy consumption by calcium-dependent ATP-ases, membrane lipid degradation and degradation of functional and structural proteins. The profile and activity of many enzyme systems will be altered, leading to numerous changes in cellular metabolism. Biochemical studies have shown that metabolism within the rheumatoid synovium is glycolytic (*i.e.* anaerobic); the activities of enzymes such as glyceraldehyde-3-phosphate dehydrogenase, glucose-6-phosphate dehydrogenase and lactate dehydrogenase will then increase. Redox imbalance is also detected, leading to the generation of reactive oxygen species

(ROS), which damage important biomolecules, such as the polyunsaturated fatty acids of the cell membrane, DNA, carbohydrates and proteins[12,17,20,22]. Intracellular pH may fall (inefficient clearance of metabolic acids from chronically hypoxic cells can reduce the mean extracellular pH). Generally, a normally oxygenated cell will maintain a pH gradient across the cell membrane of approximately 0.5 pH units when exposed to an acidic extracellular environment.[20]As a result, the intracellular pH will be higher than the extracellular pH but may fall below the physiological value. Cells in severe hypoxia, however, may be unable to maintain this pH gradient across the cell membrane; in severely hypoxic cells, therefore, the intracellular pH may fall to match the pH of the extracellular milieu. [12,17,20,22]

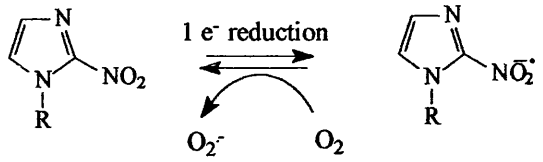
These alterations in cell physiology will alter the ability of cells to tolerate and repair injury, inducing more severe hypoxia, and will also alter the ability of cells to transport, activate, and metabolise many therapeutic drugs. [12,17-22]

3. BIOREDUCTION AND BIOREDUCTIVE DRUGS

Bioreductive drugs are activated by metabolic reduction in tumour cells to form cytotoxins. The hypothesis that hypoxic cells were offering a more favourable environment to reductive reactions than were their normoxic counterparts was developed by Lin *et al*[24], based on observations made by Hewitt and Porter[25,26] that anaerobic cultures of microbes had a lower half-wave reduction potential than did aerobic cultures. The growing cultures, under both aerobic and anaerobic conditions, became more hypoxic through crowding and their apparent redox potential became more negative. By analogy, hypoxic cells in solid tumours were suspected to exist in an environment facilitating reductive processes.

Bioreductive drugs exploit the presence of hypoxia in tumours, since oxygen (O_2) can reverse the activating step by one-electron oxidation (futile cycling, **Scheme 1**), thereby greatly reducing drug activity in most normal tissues[27-34]. Selectivity in activation of bioreductive drugs by tumour cells can also depend on the level of expression, concentration and activity of the particular reductases for which a drug can act as a substrate. These include obligate two-electron reductases, such as DT-

diaphorase, and one-electron flavoproteins, such as the various P450 isoenzymes, cytochrome P450 reductase, xanthine oxidase and xanthine dehydrogenase.[27-29]



Scheme 1: Example of futile cycling

The concentration or level of expression of these reductases seems to be often greater in tumours and hypoxic tissues than in normal tissues. However, differences [28,29] do exist in enzyme expression between tumour types.

Bioreductive drugs can be used in combination with either radiotherapy or with other chemotherapeutic agents. The drugs developed so far fall into several subgroups, including nitroheterocycles, aromatic N-oxides and various quinone-based compounds (**Figure 4**).[30-35]

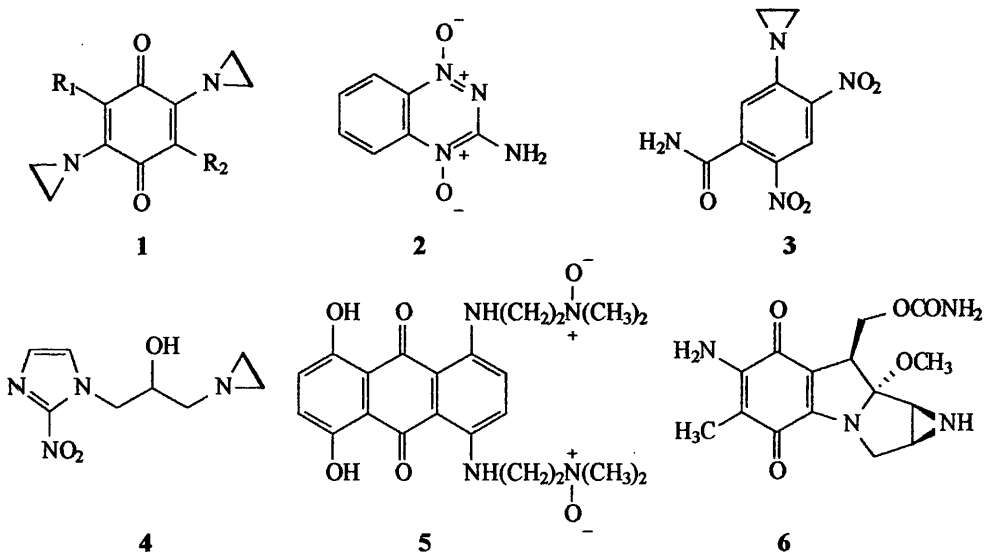


Figure 4: Chemical structure of some redox-active compounds: diaziridinyl benzoquinones (**1**), SR 4233 (tirapazamine **2**), CB 1954 (**3**), RSU 1069 (**4**), AQ4N (**5**), mitomycin C (**6**).

3.1 *THE ACTIVATING ENZYMES.*

Hypoxia and high level of specific reductive enzymes are the conditions that are needed for the bioreduction of a chosen bioreductive compound. The approach could be different; identifying the predominant enzymes in a type of hypoxic tissue could be the first step before choosing the appropriate bioreductive substrate. The enzyme systems involved in the metabolic activation pathways of quinones and nitroheterocycles include NADPH: cytochrome P450 reductase, NADPH: cytochrome b_5 reductase, NADPH:quinone oxidoreductase (NQO1, DT diaphorase), xanthine oxidase and xanthine dehydrogenase, amongst others.[27-30,35-43]

NADPH:cytochrome P450 reductase is widely distributed in human tissues and, although its main function is to reduce the various forms of the cytochrome P450, it is believed to play a key role in the reductive activation of certain toxic compounds. The activity of cytochrome P450 reductase in some tumours has been found to be significantly higher than in the normal tissue, and this has prompted extensive research on the synthesis of compounds which can be activated by this enzyme. Similarly, certain cell lines, which are resistant to drugs such as mitomycin C, show decreased levels of the enzyme. Cytochrome P450 and P450 reductase have been shown to reduce nitroimidazole, the first one to the nitro radical anion (1-electron reduction), P450 reductase is involved in the final stage, the amine formation.[35]

Studies[36-43] on the role of the other one-electron reducing enzymes in the activation of compounds in tumour cells have brought interesting results. It has been shown that the conversion of xanthine dehydrogenase into xanthine oxidase occurs in hypoxic tissues (mechanism linked to ATP depletion). Anderson *et al*[41] observed the conversion of xanthine dehydrogenase (XDH) to xanthine oxidase (XO) in two tumour types during prolonged clamping off of the blood supply to the studied tissue. It was suggested that the same phenomenon could occur naturally in chronically hypoxic cell, making it a potential marker for hypoxia. Under ischaemic conditions, depletion of ATP occurs producing hypoxanthine, which is a substrate for XDH and XO, making the two mechanisms closely linked.[41]

Two-electron reduction can be catalysed by the NAD(P)H:(quinone acceptor) oxidoreductase (quinone reductase, DT-diaphorase)[28,29,38,39]. There is evidence that the levels of this enzyme are increased in some tumours. It is believed that the two-electron reduction of a compound is a protective mechanism. This is because extensive studies with simple quinones, such as menadione, have shown that inhibition of the diaphorase system potentiates the cytotoxicity of the quinones. It is shown that, by removing the quinone *via* the two-electron reaction, less quinone is available for the more damaging one-electron reaction (because of the subsequent production of superoxide radicals) or for the quinone-mediated depletion of intracellular thiols[38,39].

$$Q + 2\,RSH \longrightarrow QH_2\text{-}RSSR \qquad (Equation\ 1)$$

There are several other examples in which the diaphorases can be instrumental in preventing the potential mutagenicity or carcinogenicity of different compounds.
However, studies[38,39,44] have shown that some compounds which can undergo reaction, such as mitomycin C and AZQ (diaziquone, **1**, $R_1 = R_2 = NHCOOCH_2CH_3$), can be activated by diaphorases to form cytotoxic species.
Further complications can arise when compounds which have the capacity to be reduced by the one-electron reducing enzymes, come into equilibrium with the two-electron reduced form.

$$D^{\cdot-} + D^{\cdot-} \rightleftharpoons D^{2-} + D \qquad (Equation\ 2)$$

Even in situations where equilibrium is favoured towards the right-hand side, free radicals could be produced during the aerobic oxidation of the D^{2-} forms.

$$D^{2-} + O_2 + 2H^+ \longrightarrow D + H_2O_2 \qquad (Equation\ 3)$$

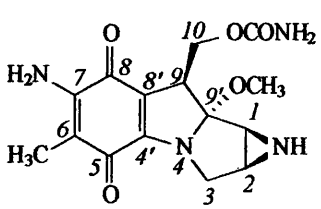
In general, the rate of aerobic oxidation of the two-electron reduced species, in the absence of suitable catalysts, is much slower than that for one-electron reduced

species but the overall net reaction can be accelerated for some hydroquinones by superoxide dismutase as observed by Cadenas *et al*[36].

Bioreductives can therefore undergo interrelated one-electron or two-electron reductions[28,29]. The question arises as to which enzyme process within a cell type will dominate for a particular drug. The relative activities of the different enzymes and the availability of their co-factors is a major consideration. This approach has led to suggestions that the relative enzyme activities in the hypoxic tissues of individual patients should be determined even before the start of drug treatment.

3.2 *MITOMYCIN C*

Mitomycin C is a clinically important antibiotic and antitumour agent isolated from *Streptomyces caespitous*. This quinone-based alkylating agent, functions by inhibiting DNA replication. It contains, in addition to a fused aziridine ring, two potentially reactive centres, a C10 carbamate moiety and a para-quinone[44]. MMC was shown to exhibit hypoxia selectivity.[44] FR900482 possesses the same carbon framework as MMC. Isolated from *Streptomyces sandaensis*, it is also known as an antitumour antibiotic acting by DNA bis-alkylation.[45]



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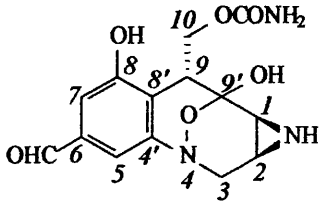

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Figure 5: Structure of mitomycin C (**6**) and natural product antitumour antibiotic FR900482 (**7**).

MMC (**Figure 5**) requires reductive activation, as does FR900482, but the mechanisms of activation leading to the active intermediate (structurally similar 7-aminoleucoaziridinomitosene) responsible for DNA alkylation are different.[46]

Detailed structures of the major MMC-DNA adducts (mono- and bi-functional) have been elucidated. The main covalent binding sites are at the aziridine C-1 and the carbamate C-10 positions, which link with guanine at the N-2 positions in CG-GC rich regions (**Figure 6**).[44-49]

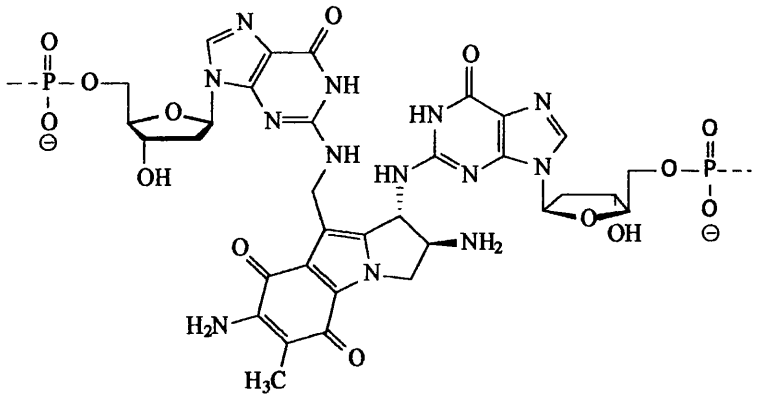

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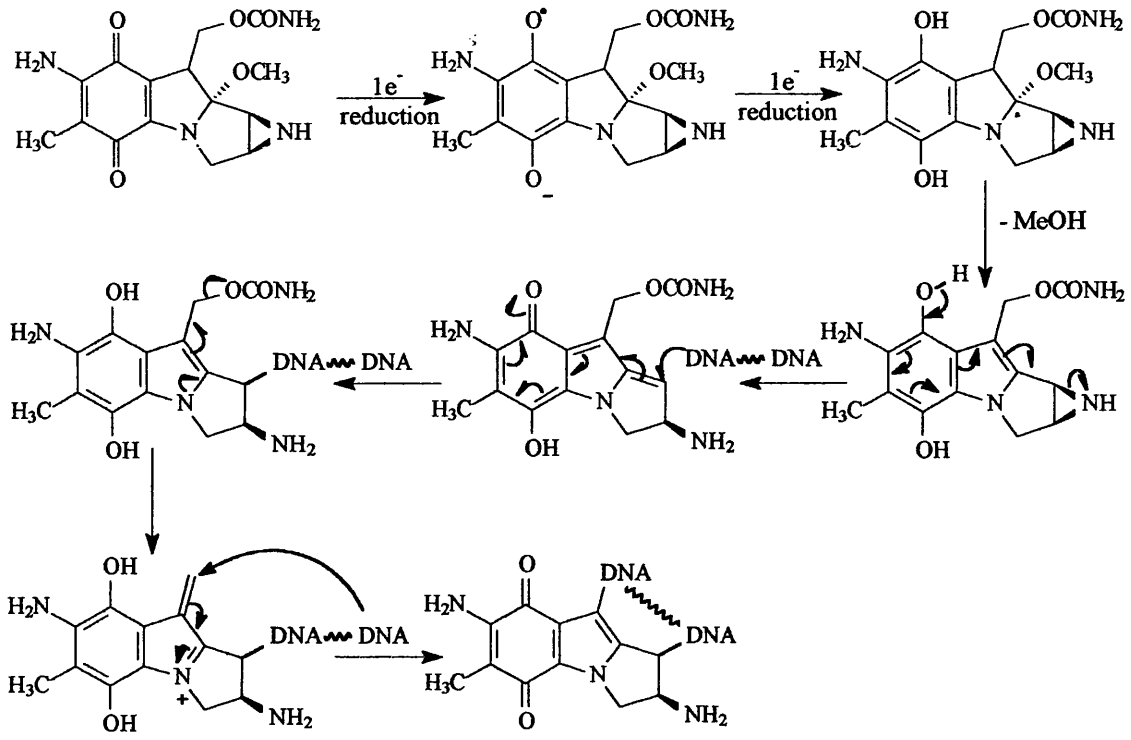
Figure 6: The structure (**8**) proposed[44,47] for the DNA-mitomycin C-DNA cross-link, showing the links between C-1 of mitomycin C and N-2 of guanine and between C-10 of mitomycin C and N-2 of guanine'.

Structure-activity studies on the toxicity of a number of mitomycin analogues towards human tumour cell lines have shown a significant correlation between the reduction potentials of the analogues and their cytotoxicity[44]. It has long been assumed that MMC was only activated by the one-electron reducing enzymes such as cytochrome P450 reductase or xanthine oxidase (one-electron reduction potential E^1_7: -310 mV). The resulting semiquinone radical is unstable in air, presumably implying great hypoxia selectivity, but it was observed that the toxicity of MMC semiquinone radical towards hypoxic cells was also accompanied by a relative degree of toxicity towards oxic cells.[44]

It is now believed that the covalent binding of MMC to DNA occurs through the two-electron-reduced form, which can be produced by one- or two-electron systems. It has been shown that MMC can be activated to the cytotoxic form by DT-diaphorases in the presence of oxygen and the cytotoxicity can be related to the activity of the diaphorase in cell lines. The proposed mechanism is that the MMC binds to the enzyme and undergoes a specific two-electron reduction to the active form of the drug

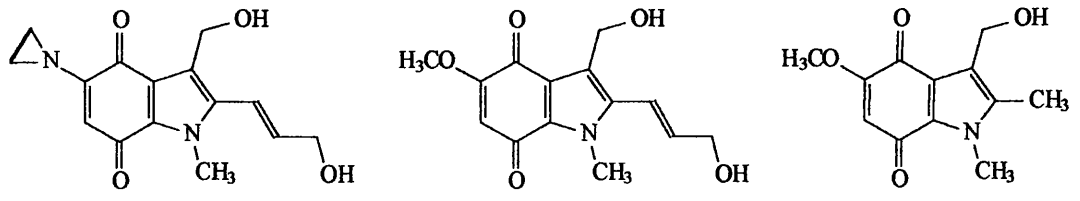
which can then interact with DNA. Intracellularly, these processes would have to take place in close proximity to nuclear DNA[44-47] (**Scheme 2**).



Scheme 2: Proposed activation mechanism of mitomycin C.[47]

3.3 *MITOMYCIN C ANALOGUES.*

The strong interest in the antitumour potential of the indoledione class of bioreductively activated cytotoxic drugs has led to the synthesis of a large number of MMC analogues[50-64]. Compounds of note include the diols EO9 and EO7, of which EO9 was evaluated in clinical trials.



9 **10** **11**

Figure 7: Structures of the indolediones EO9 (**9**); EO7 (**10**); and 1,2-dimethyl-3-(hydroxymethyl)-5-methoxyindole-4,7-dione (**11**).

Related mitosenes (MMC analogues with an aromatic 5-membered ring, **Figure 7**) also show much improved properties over the naturally occurring mitosanes (non-aromatic 5-membered ring) such as MMC and many analogues have been produced over the past 20 years[52,53].

3.3.1 *Cyclopropamitosenes*

In recent years, other classes of indolediones, such as the mitosene-based cyclopropamitosenes[53-56] and the non-fused cyclopropylindolediones, have also been developed and these compounds have also emerged as highly potent compounds, the latter showing efficacy in animal tumours. The earlier cyclopropamitosenes resembled the MMC structure (**Figure 8**) but the latter compounds also had features that were similar to EO9, namely the 5-aziridinyl substituents and the 3-hydroxymethyl group (**Figure 10**)[57]. It was suggested[54] that the cyclopropyl ring at the C-2 position may be opened through the activation mechanism (utilising one-electron reductases) and that this might be involved in the enhanced hypoxic cytotoxicity observed. This has now been further explored.[58,59]

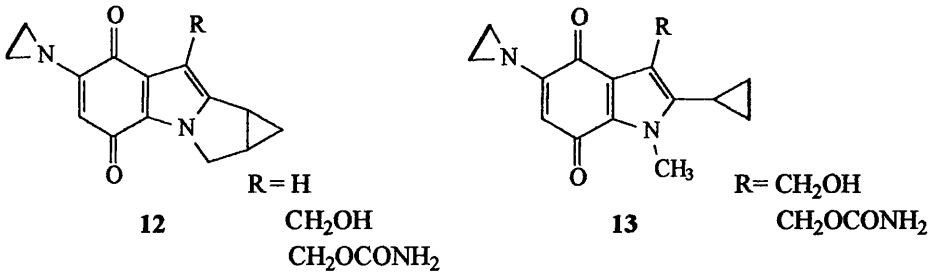
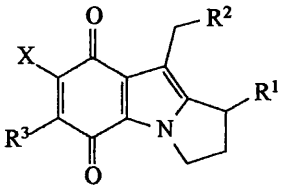


Figure 8: Cyclopropamitosene derivatives.

3.3.2 *Other mitosenes.*

A wide range of mitosenes based on the MMC substructure have been synthesised by many groups[60-64]. Mitosenes possessing at least one good leaving group at the C-1 and/or the C-10 position showed greater toxicity than MMC. Other analogues have been synthesised which possessed poor leaving groups and, here, only low activity

was observed (**Figure 9**)[53-54]. Under hypoxic conditions, the toxicity of the mitosenes in V79 cells was increased, as compared with aerobic toxicity. DNA cross-linking experiments demonstrated the need for efficient leaving group at positions activated by reduction. The penetration of these compounds into the tumour cell (related to lipophilicity) seems to be a more important factor than structural variation, although the absolute configuration of the C-1 of some of the synthetic chiral mitosenes, appeared to be important for enzymatic reduction and DNA cross-linking.



14

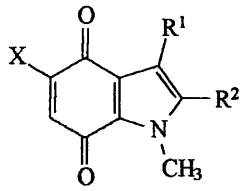
$X = OCH_3$	$R^1 = H$	$R^2 = H$	$R^3 = CH_3$
OCH_3	$OCOCH_3$	$OCOCH_3$	CH_3
Az	$OCOCH_3$	$OCOCH_3$	CH_3
OCH_3	H	$OCONH_2$	H
Az	H	$OCONH_2$	H

Figure 9: Mitosene derivatives.

3.3.3 *EO9 and analogues.*

Analogues of EO9 and their regioisomers have been synthesised in order to investigate the relative contributions of substituents at the C-2, C-3 and C-5 positions of the indole ring with respect to the cytotoxic effects (**Figure 10**). Replacement of the 5-aziridinyl with a methoxy moiety (EO7) showed a marked loss of potency under both aerobic and hypoxic conditions. Replacement of the 5-aziridinyl with a 2-methylaziridinyl moiety showed a slight loss of potency overall but more so under aerobic conditions; further reductions were observed with greater aziridine substitution[50]. These compounds were also highly hypoxia-selective agents but were less potent than EO9[57]. Removal of the C-2 substituent of EO9 resulted in a compound that showed similar potency under both aerobic and hypoxic conditions. When the C-3 was removed, a loss of potency was observed. As a result of this study, it was suggested that the aziridinyl substituent was the predominant factor in producing the observed cytotoxicity, followed by the C-3 hydroxymethyl group, which was thought to be involved in alkylating processes. Replacement of the 5-aziridine group with 2-methylaziridine gave a compound (EO8) with a greater hypoxic cytotoxicity ratio (HCR), which was accredited to its lesser aerobic potency

(due to the fact that EO8 is a poorer substrate for DT diaphorase than EO9), rather than hypoxic toxicity, in both rodent and human cell lines[57].



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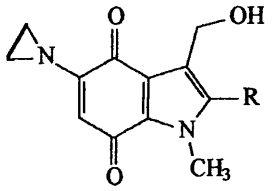
X =	R^1 =	R^2 =	
Az	$CH=CHCH_2OH$	CH_2OH	**EO9**
2-MeAz	$CH=CHCH_2OH$	CH_2OH	**EO8**
OCH_3	$CH=CHCH_2OH$	CH_2OH	**EO7**
Az	H	CH_2OH	
Az	$CH=CHCH_2OH$	H	
Az	CH_3	H	

Figure 10: EO9 analogues.

Other indoledione analogues similar to EO9 have been synthesised in which the C-2 position was substituted with various alkyl groups[50]. With the exception of EO9, a trend was observed in which increasing bulkiness at the C-2 position resulted in a decrease of both aerobic and hypoxic potency (**Figure 11**). This indicated either that the incoming DNA (or other nucleophilic species) at the C-3 position was hindered or that reductase enzyme specificity was being altered. Bulky C-2 substituents may also influence the ease of formation of the iminium species, in which the 3-indolyl CH_2 must become sp^2 hybridised and achieve planarity. The straight alkyl side-chain of EO9 may have a lesser effect in this respect. However, the potency of EO9 suggests that other processes must be involved in enhancing its hypoxic cytotoxicity, such as the reductive chemistry of the prop-2-enyl moiety for example.



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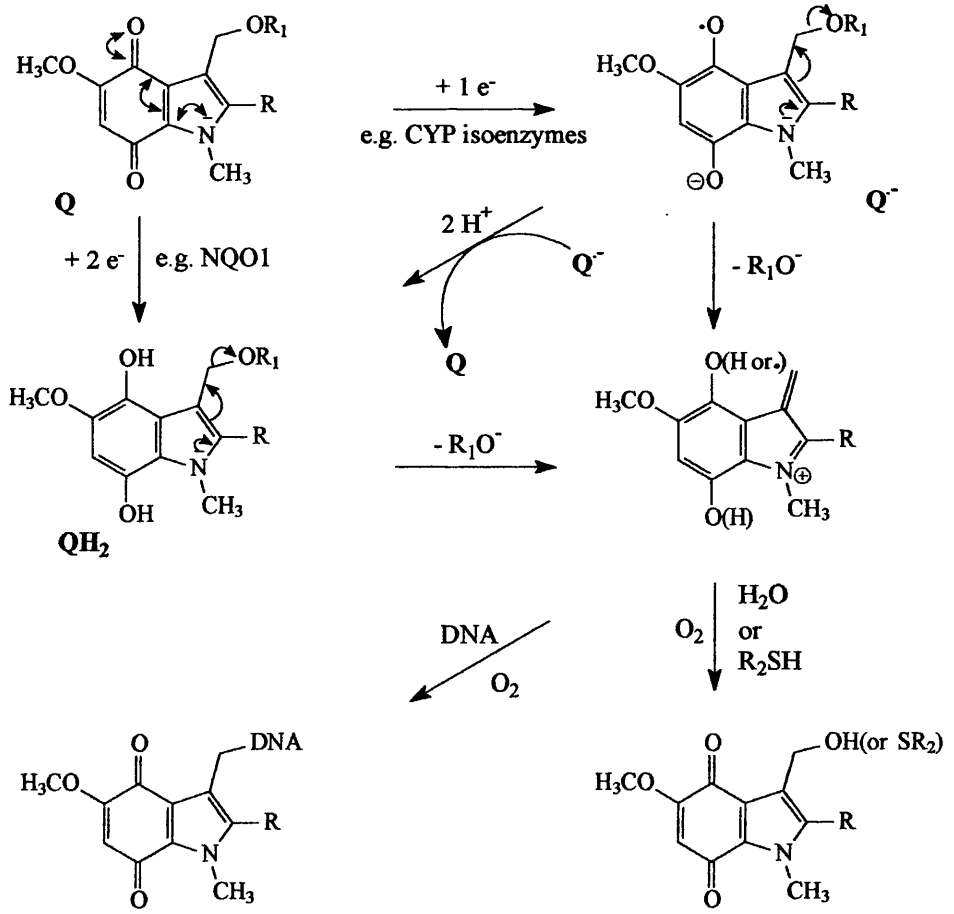
R = H
CH_3
$CH(CH_3)_2$
Cyclohexyl

Figure 11: Indoledione derivatives.

Recently, a series of 2-alkyl and 2-cycloalkyl indolediones has been evaluated[50] as bioreductively activated cytotoxins that show *in vivo* activity against solid tumours with defined hypoxic fractions and are also targeted against tumour tissues rich in the required activated enzymes.[50]

Of particular importance is the potential for 3-indolyl carbinyl substituents in derivatives of (**11**) to undergo a "retro-Michael" elimination process upon reductive activation, through the participation of the 1-nitrogen lone pair of electrons which are deactivated in the quinone parent prodrug (**Scheme 3**). The resulting iminium species, distinct from the quinone methide generated by simpler quinones, is then a potential cytotoxic species *via* DNA-alkylation or other critical biomolecules, or other cell-damaging species. These compounds have the potential to release a variety of drugs in a reductive environment, and may thus be designed to give a secondary effect in addition to the cytotoxic iminium derivative formed.[55-57]

This process is rendered possible because of the conjugation between the lone pair of electrons on the nitrogen atom, the double bond on the aromatic 6-membered ring and the double-bonded oxygen. This kind of pattern is recognised as a vinylogous amide.

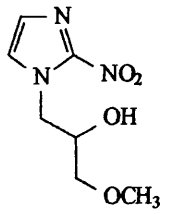


Scheme 3: Reductive activation pathways for indolediones leading to elimination of leaving group and trapping of the resulting iminium intermediate.

3.4 *NITROIMIDAZOLES.*

A second category of bioreductive agent is that of the nitro(hetero)arenes, the prototypes of which are misonidazole and metronidazole (**Figure 12**). This category of compounds arose from the group of agents known as radiosensitisers. The neurological toxicity of misonidazole and other nitroimidazoles limit their clinical effectiveness, therefore, new agents are being produced which maintain this hypoxic cell toxicity and exhibit decreased neurotoxicity.

It has been recognised for several years that most of the biological properties of nitroheterocyclic compounds are determined by their ease of reduction. The nitroimidazoles were originally investigated for their ability to act as radiosensitisers in hypoxic cells. However more recent studies have shown that some of these compounds may also be promising as potential chemotherapeutic agents.[28,32,33,35,65-67]



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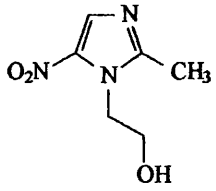

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Figure 12: Structures of misonidazole (**17**) and metronidazole (**18**).

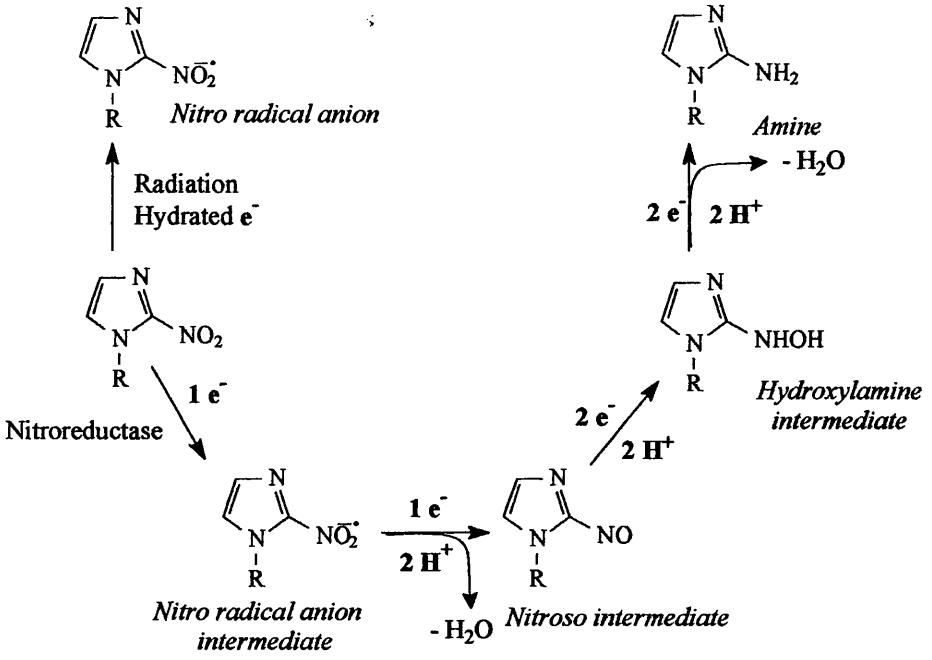
When nitroimidazoles are incubated with anaerobic cell extracts, nitro radical anions ($RNO_2^{\cdot-}$) can be produced[32]. In normoxic cells, these radicals are normally reoxidised to the parent drug in the presence of oxygen due to the equilibrium :

$$D^{\cdot-} + O_2 \rightleftharpoons D + O_2^{\cdot-} \qquad (Equation\ 4)$$

However, in the absence of oxygen, the nitro radical can dismutate to form DNA damaging species :

$$D^{\cdot-} + D^{\cdot-} \rightleftharpoons D^{2-} + D \qquad (Equation\ 2)$$

Further reduction leads to the generation of intermediate metabolites and to the amine Toxicity is believed to be due to the intermediate metabolites (**Scheme 4**).



Scheme 4: Proposed reduction scheme of 2-nitroimidazoles.

RSU 1069 (**Figure 1**) showed many improved properties, compared to misonidazole, and has been widely studied. Many other analogues are being produced to improve the selectivity and cytotoxicity of the nitroimidazoles.[68]

3.5 *NITROHETEROCYCLIC TRIGGERS.*

Much effort has been expended on development of radiosensitisers with electron affinity and bioreductively activated cytotoxins for selective therapy of hypoxic tissue, and of a variety of prodrugs to deliver cytotoxins selectively to tumours. Substituted 2-nitroimidazoles are known to be selectively retained in hypoxic tissue by reductive metabolism[66]. The exploitation of the physiological difference in concentration of molecular oxygen between normal and hypoxic tissue leads to the design of biologically inactive prodrug systems which upon selective bioreduction in hypoxic tissue would release known therapeutic drugs only in that tissue.

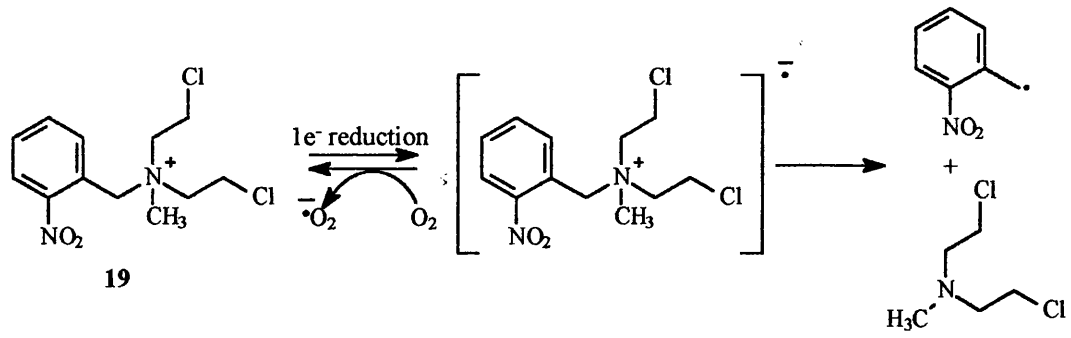
Bioreductively triggered release systems based on 2-nitroarylamines and 4-nitrobenzyloxycarbonyl prodrugs have been reported, and reductive elimination of leaving groups have been studied involving 2-, 4- and 5-nitroimidazoles, 5-nitrofurans, 5-nitropyrrole and nitrobenzene.

Extensive work on nitrobenzyl systems for cancer therapy has been reported. Substance such as nitrobenzyl quaternary salts and nitrobenzyl mustards gave encouraging results[69-74]. Those mustard-based hypoxia-selective cytotoxins (HSC) were designed following the same pattern. They consisted of a trigger unit (hypoxia selective (oxygen-sensitive) and undergoing the reductive process), an effector (activated following reduction of the trigger) and a linker which connects the two other domains and communicates the change (**Figure 13**).[74]

Figure 13: Denny's concept for the design of anti-cancer prodrugs.[74]

Amongst other systems (nitro-deactivated aniline mustards, cobalt(III) complexes of aliphatic mustards and nitrobenzyl quaternary mustards), nitrobenzyl quaternary mustards due to their ability to release a leaving group following one-electron reduction, seemed to have the quality required to be used for the design of HSCs.[69-76]

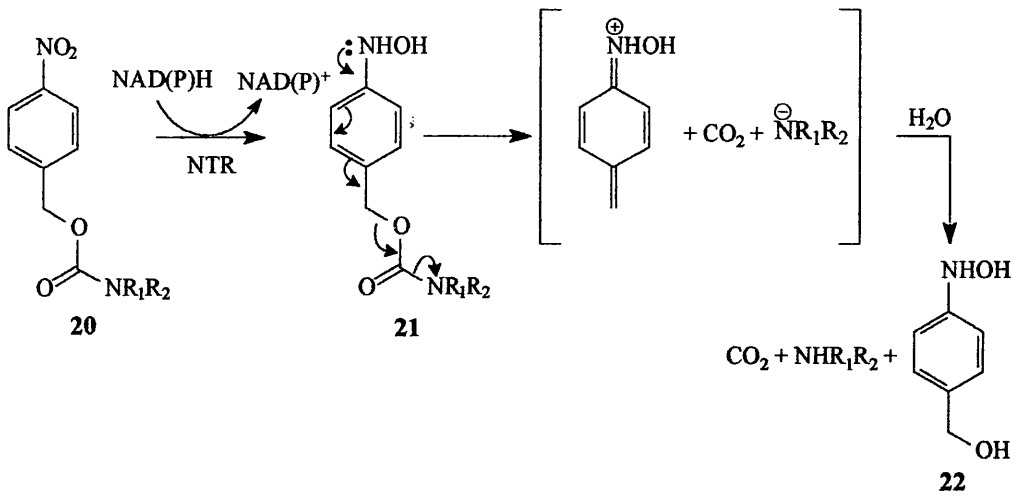
Nitrobenzyl radicals, resulting from the fragmentation of nitrobenzyl halides, proved to be cytotoxic, nearly equally to both oxic and hypoxic cells. In the case of nitrobenzyl quaternary salts, hypoxia selectivity was improved and less toxicity was attributed to the more stable benzyl radical. Studies on nitrophenyl quaternary mustards (**Scheme 5**) also permitted to identify some important physicochemical properties influencing the efficiency of the HSCs. Aqueous solubility and one-electron reduction potential are critical properties for the activity of the bioreductive compounds.[74]



Scheme 5: Reductive fragmentation of a typical nitrobenzyl quaternary mustard.

The one-electron reduction potential (E^1_7) of nitro aromatics, and most bioreductive compounds should be within the range –250 to –500 mV for selective enzyme-catalysed reduction to take place. Aqueous solubility can be influenced by the introduction of acidic or basic groups. The introduction of basic functionalities (cationic charges) induced an increased potency of some bioreductive drugs in cell cultures together with improved solubility. Positively charged compounds (**Scheme 5**) gave poor biological results. The lack of activity *in vivo* could be due to the trapping effect of low pH cellular organelles or to the reversible DNA binding also observed for positively charged compounds. In both cases, the decreased concentration of free drug reduced the rate of passive diffusion through the tissues to the site of activation. The introduction of anionic groups (weak acids) ensured a better cellular uptake in low pH environment.

Following these results, 4-nitrobenzyl carbamate prodrugs were produced for gene-directed enzyme prodrug therapy (GDEPT) [31] and antibody-directed enzyme prodrug therapy (ADEPT) [31]. This concept involves the use of tumour-specific endogenous enzymes, which are capable of activating bioreductive drugs, by deliberately introducing foreign enzymes into the cell population. ADEPT implies the use of tumour-specific antibodies in order to locate the foreign enzyme; in the case of GDEPT a foreign gene is used in order to generate the enzyme selectively. Assessment *in vitro* showed release of the drug moiety. Activation of the nitro group occurred *via* the conjugated effect of an oxygen-insensitive nitroreductase (NTR, from *Escherichia coli* B) and NADH or NADPH to give the hydroxylamine derivative (**Scheme 6**)[31,77-79].



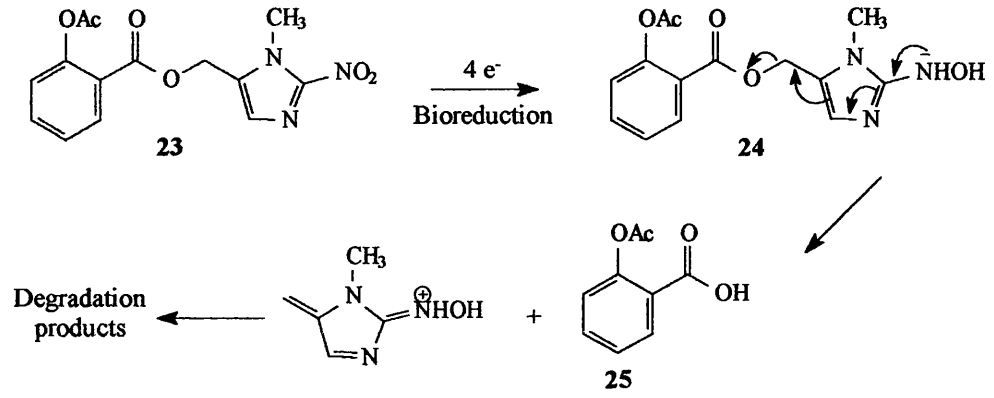
Scheme 6: Reduction of nitrobenzyl carbamate by NTR.

A number of 4-nitrobenzylcarbonyl derivatives including actinomycin D, mitomycin C, an endiyne, amino-seco-cyclopropylindoline derivatives and tallimustine analogues have been shown to be substrates for the enzyme, but with a different degree of activation, pH and leaving group effect did influence the rate of release of the effector.[72,77-79]

In the family of the 5-membered-ring nitroheterocycles, 2-nitroimidazole is amongst the most studied. 2-Nitroimidazole derivatives are used and studied for many cancer related therapeutic purposes but mainly for radiotherapy and as hypoxia markers. Their redox potential is very favourable for potential bioreductive activation by endogenous enzymes (E^1_7 = -389 mV for 1-alkyl-2-nitroimidazole)[80]. A number of 2-nitroimidazole-derived prodrugs have been synthesised.

Hay *et al*[81] reported the synthesis and evaluation of a 2-nitroimidazole carbamate prodrug of amino-*seco*-CBI-TMI for use in ADEPT and GDEPT. The reduction of the prodrug using a nitroreductase from *Escherichia coli* B in conjunction with NADH or NADPH showed the release of the amine effector, together with good hypoxia selectivity and cytotoxicity on two different cell lines.

Everett *et al*[82] used 2-nitroimidazole to generate a series of prodrugs using aspirin or salicylic acid as the effector moiety. The drug counterpart was linked through the (imidazol-5-yl)methyl position of the trigger unit. Release of the effector drugs was observed after activation by $CO_2^{\cdot-}$, a model one-electron reductant generated radiolytically. The prodrugs exhibited improved water solubility compared to quinone-based prodrugs, partly due to the substitution at the imidazol-1-yl position. Studies showed that the release of the drug moiety occurred after 4-electron reduction, leading to the hydroxylamine derivative[80-82].The rate of drug release was still slower than for the 4-nitrobenzyl analogues.(**Scheme 7**)

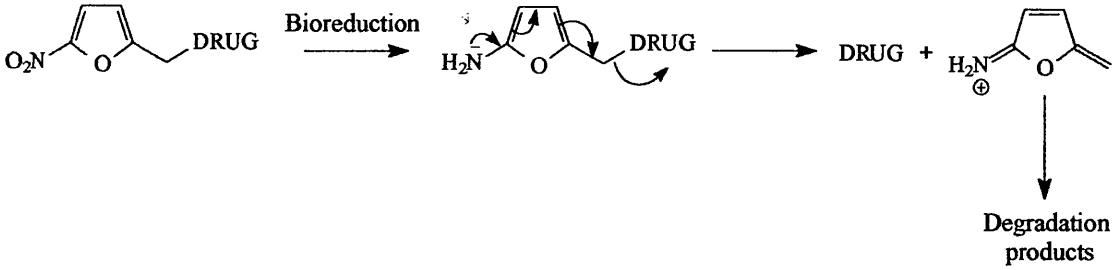


Scheme 7: Proposed mechanism for the elimination of aspirin from the bioreduction of the 2-nitroimidazole prodrug.[82]

Parveen *et al*[80] used a similar prodrug system for the release of 5-bromoisoquinolinone, a PARP inhibitor. The secondary amide of the isoquinolinone moiety, main site for activity, was masked by 2-nitroimidazol-5-ylmethyl. The chemical reduction of the nitroimidazole to the amine created an increase in electron density of the π-system, resulting in the expulsion of the drug moiety.

5-Nitrofurans have a relatively high redox potential which makes them more favoured for bioreductive activation (E^1_7 = -325mV for 2-methyl-5-nitro-N-(prop-2-enyl)furan-3-carboxamide) than 2-nitroimidazole; they are activated by enzymes such as cytochrome P450 reductase[83-85]. Compounds with a one-electron reduction potential closer to the redox potential of oxygen (-180 mV) are more likely to gain an electron and are therefore more oxidising. They are consequently less hypoxia-selective and

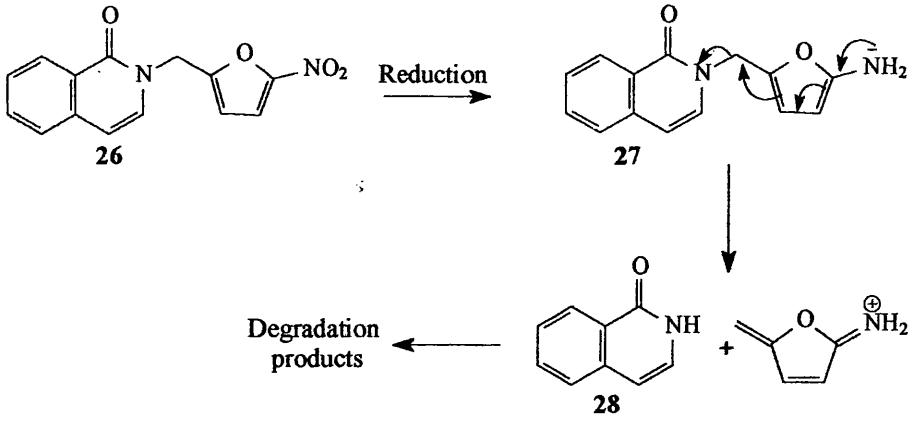
are often more toxic. On the other hand, compounds with more negative E^1_7 are less oxidising and less likely to gain an electron (**Scheme 8**).



Scheme 8: Proposed mechanism for the bioreductively triggered release of drugs from nitrofuranylmethyl prodrugs.[83-85]

Naylor *et al*[85] synthesised a series of nitrofurancarboxamides which were evaluated as radiosensitisers and bioreductively activated cytotoxins but showed poor hypoxia selectivity and increased toxicity. The carboxamide side chain in these prodrugs was described as influencing the redox potential markedly. Compounds with a one-electron reduction potential, E^1_7 = -335 mV, *i.e.* slightly lower, showed increased hypoxia selectivity.

Nitrofurans were used as the trigger unit by Berry *et al*[83]. A 5-nitrofuranyl-2-methylene was linked directly to the 2-position of isoquinolinone and through a carbamate to a carboranylpropylamine. Biomimetic reduction of the nitro group initiated the expulsion of the isoquinolinone drug moiety (**Scheme 9**) and of the carboranylpropylamine, due to the increase of electron density of the π-system following reduction.[83]



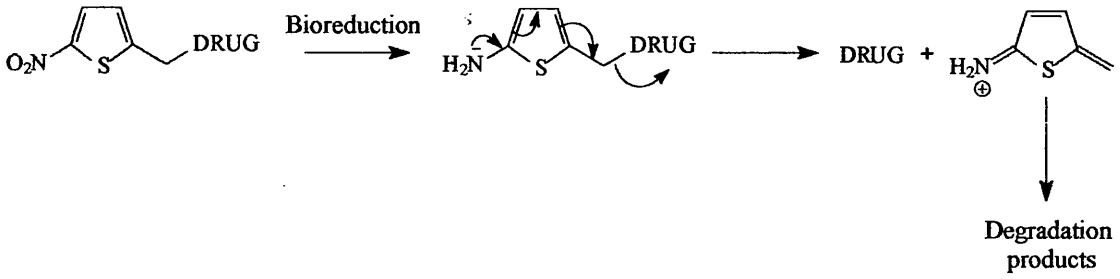
Scheme 9: Reductively activated release of isoquinolinone from nitrofuran based prodrug[83].

Mahmud *et al*[84] reported the preparation of a nitrofuran-based prodrug obtained from the condensation of 5-nitrofuran-2-carboxaldehyde with two different diols, giving the corresponding cyclic nitrofuranyl acetals. Biomimetic reduction of these prodrugs with sodium borohydride and palladium on carbon induced the release of the diols. Reduction of the nitro group was rapid (1 to 5 min) but the maximum yield of diols was only obtained after an hour.

Nitrothiophene prodrugs were prepared and studied as 5-carboxamide derivatives bearing N-(ω-aminoalkyl) side chains, designed to be evaluated as radiosensitisers and potential bioreductively activated cytotoxins[86]. The difference in one-electron reduction potential between the 2- and 3-nitrothiophene derivatives was of 200 to 250 mV, the 2-nitro being more oxidising (E^1_7 = -271mV). Their *in vitro* potency as radiosensitisers was greater than that observed for misonidazole and 2-nitroimidazole derivatives. The neurotoxicity and lack of hypoxia selectivity of the compounds was attributed to the influence of the side chain.

The results obtained for the nitrobenzyl prodrugs, but especially for the 2-nitroimidazole and 2-nitrofuran prodrugs suggested the interesting potential of nitrothiophene to act as a bioreductive delivery tool. With a more negative E^1_7 than nitroimidazole it could be expected to be more hypoxia selective and less toxic.

The toxicity of the aminofuran moiety resulting from the reduction of the prodrugs synthesised by Berry *et al*[83] has not been yet evaluated[87].



Scheme 10: Speculative scheme of eventual bioreductively triggered release of drugs from nitrothienylmethyl prodrugs.

4. DRUGS FOR DELIVERY IN RHEUMATOID ARTHRITIS

4.1 *PARP INHIBITORS.*

Poly(ADP ribose) polymerase (PARP) is a nuclear enzyme which is activated by single DNA strand breaks (The enzyme N terminus has two zinc finger motifs which bind to the cleaved DNA chain). It then binds NAD^+ (β-nicotinamide-adenine dinucleotide), *via* C-terminal domain and catalyses the synthesis of poly(ADP)ribose. A side product of this process is nicotinamide, which has been recognised as an inhibitor of PARP (studies have shown that this inhibition is weak and non-specific). Extensive DNA damage leads to over stimulation of PARP, which results in depletion of the "cellular energy currency" molecules NAD^+ and ATP and to cell death. This is known as the "suicidal hypothesis" and ensures that no unwanted mutations arise.[88]

PARP is then thought to play an important role in maintaining genomic integrity and facilitate chromatin structural changes during DNA repair. It somehow influences gene expression, DNA replication, rearrangement, differentiation and mutagenesis. Isoforms of the enzyme have been discovered with similar catalytic domains. The exact functions of PARP are still unclear but the study of different classes of the enzyme inhibitors allowed the identification some of these functions[88].

Reactive oxygen species (ROS), such as superoxide, hydroxyl radicals and hydrogen peroxide have shown to be important inducers of PARP activity. Inflammation and rheumatic diseases exhibit cellular redox imbalance and hyperactive immunity which trigger increased levels of ROS and nitric oxide. Injuries in RA caused by hypoxia/reperfusion sequences induce high levels of PARP. Studies on PARP knock-out rodents with induced hypoxia or ischaemia (where ROS are shown to be present and cause tissue damage) presented decreased tissue damage.

An investigation of the role of PARP in rheumatoid arthritis was carried out by Miesel *et al*[89]. RA was induced in rodents by using K_3CrO_8. The *in vivo* decay of the potassium peroxochromate to superoxide, singlet oxygen, hydroxyl radicals, hydrogen peroxide and CrO_4^{2-} caused chronic inflammation and arthritis, mainly by depletion of intra- and extracellular antioxidants and conjugated inhibition of the main anti-oxidases, such as superoxide dismutase. Nicotinamide **29**, one of the first substances tested for PARP inhibition[88], was selected as the inhibitor to determine the anti-arthritic activity. It proved to reduce K_3CrO_8-induced RA by 35% in rodents and inhibited the phagocytic generation of ROS. Nicotinamide and 3-aminobenzamide, another type of PARP inhibitors, were shown to decrease TNFα-mediated cytotoxicity which triggered the reduction of ROS production (TNFα influences NADPH oxidases leading to the increased production of ROS)[88,89]. Other inhibitors were developed to give more potent substances against ROS, such as GPI 6150, (1,11b-dihydro-[2*H*]benzopyrano[4,3,2-*de*]isoquinolin-3-one)[90]. This substance proved to be potent against PARP in models of focal cerebral ischaemia, traumatic brain injury, regional myocardial ischaemia, streptozotocin-induced diabetes, septic shock and arthritis. GPI 6150 was reported to protect a specific cell line against hydrogen peroxide-induced cytotoxicity by preventing PARP activation and depletion of its substrate, NAD^+. No side effects, such as impairment of repair and expression of damaged DNA, were reported. The substance exhibited low potency towards mono-ADP-ribosyltransferase and showed no selectivity between PARP isozymes[90].

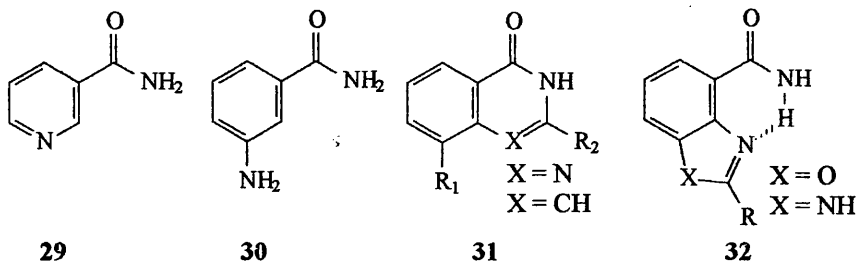


Figure 14: Structures of PARP inhibitors mimicking the nicotinamide moiety of the substrate: nicotinamide, 3-aminobenzamide, 3,4-dihydroisoquinolin-1-ones, 2,8-disubstituted quinazolin-4-ones, benzoxazole-4-carboxamides and benzimidazole-4-carboxamides[91-94].

The results of these studies suggest the important role of PARP in the inflammatory cascade observed in RA. However, nicotinamide and 3-aminobenzamide (**Figure 14**) have been shown to be less potent than the more recently tested substituted isoquinolin-1-ones. Suto *et al*[92] and Watson *et al*[93] reported the synthesis and evaluation of 5-substituted isoquinolin-1-ones, namely 5-bromo and 5-iodoisoquinolinone. Their potency against PARP was superior to that of the 3-substituted benzamide family *in vitro*. Another member of the isoquinolinone family, 5-aminoisoquinolinone, reported by McDonald *et al*[94] showed high activity for PARP inhibition in human cardiac myoblasts. It also diminished the multiple organ injury and dysfunction caused by severe haemorrhage and resuscitation (which in turn caused tissue ischaemia, ROS and NO production, DNA damage and PARP activation)[94,95].

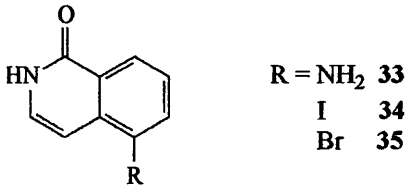


Figure 15: Structure of 5-aminoisoquinolin-1-one, 5-iodoisoquinolin-1-one, 5-bromoisoquinolin-1-one.

The interesting results given by 5-substituted isoquinolin-1-ones, together with the evidence that PARP inhibition reduces inflammatory conditions observed in RA, justify the choice of compounds **33**, **34** and **35** as drug moieties for the present study.

4.2 *GLUCOCORTICOIDS.*

This category of drugs has now been used for 50 years in the treatment of rheumatic diseases. Their profound anti-inflammatory activity is balanced by their serious risk of side-effects in the long term.

They exhibit complex anti-inflammatory and immunomodulatory effects. They inhibit migration of leucocytes to sites of inflammation and interfere with the function of leucocytes, endothelial cells and fibroblasts. They suppress production and release of factors involved in the inflammatory response such as cytokines, prostaglandins and leukotrienes. Although the effect of corticosteroids is mainly inhibitory, they also increase the level of expression of some cytokines or induce anti-inflammatory factors (**Table 2**).[2]

Inflammatory factors	Cytokines	Chemokines	Adhesion molecules	Receptors Others
Inhibition	IL1,2,3,4,5,6,11,12,13-TNFα-GMC-SF	MCP-1,3,4 IL8, MIP-1α, Eotaxin,	ICAM-1, E-selectine	GR, NK-1,2 IL-2,3,4,12R INOS, COX-2,
Induction	IL 10			IL-1R type I&II, IL-6R Lipocortin 1

Table 2: Cytokines and anti-inflammatory factors induced or inhibited by corticosteroids.

The side effects observed when using corticosteroids can be explained from the fact that most cells in all the different types of tissue in the body possess specific receptor for corticosteroids known as glucocorticoid receptors (GR). The glucose and lipid metabolism are affected as well as the skeletal tissues, CNS, renal, immune and cardiovascular systems. This is illustrated by phenomena of hyperglycaemia, weight gain, depression, psychosis, osteoporosis, hypertension, skin disorders, glaucoma, cataracts, oedema, thromobosis, gastrointestinal disorders, hypersusceptibility to

infection and many others. The mechanism of action of glucocorticoids can give rise to transcription or repression of the targeted gene[2,4,5].

The glucocorticoid binds to an intracellular receptor (GR) which controls gene expression by hormone dependant regulation of transcription. This regulation can be positive or negative. Since steroids are highly lipophilic, they cross the cell membrane easily to reach the receptor (cell membrane binding sites have been described and are thought to be involved in transport). The activated receptor then binds to a specific region of DNA called HRE (hormone response element) to regulate the transcription of adjacent genes. Activation of the receptor *via* hormone binding triggered a conformational change of the receptor that allows it to regulate transcription. The activated hormone-receptor complex translocates from the cytosol to the nucleus and binds to DNA in a dimeric form (**Figure 16**).[2,4,5]

The HRE is normally upstream from the promoter of the target gene; binding of the activated complex to the HRE either stabilises (positive regulation) or interferes (negative regulation) with binding to the accessory transcription factor. This step is essential for RNA polymerase II to bind tightly to the promoter and initiate transcription. The receptor is made of a single polypeptide chain containing two highly conserved regions. The first one is the DNA binding region; it is cysteine-rich and has two zinc binding fingers for HRE specificity. The second is the carboxy-terminal portion, which contains the hormone-binding and dimerisation domains. The amino-terminal region and the area between the two conserved regions are not essential for DNA binding but may have an influence. However, these areas interact with the transcription factor and target the receptor to the nucleus.[2,4,5]

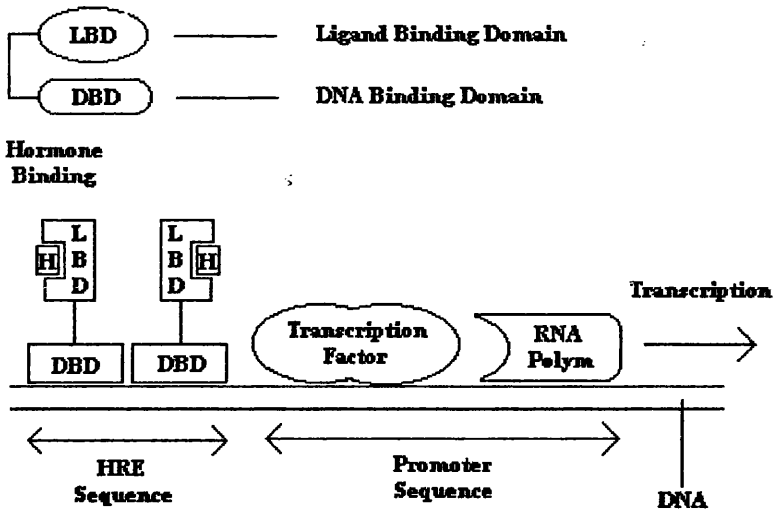


Figure 16: Binding of the Hormone (H) causes translocation of the protein to the nucleus, dimerisation and formation of a complex of proteins at the promoter. Members of the steroid receptor family bind to DNA at the HRE (Hormone Response Element) and facilitate or inhibit the formation of active transcription complex at the promoter.

Some of the corticosteroids commonly used for anti-inflammatory purposes are betamethasone, cortisone acetate, dexamethasone, hydrocortisone, methyl-prednisolone, prednisolone, prednisone *etc.* In rheumatoid arthritis, prednisolone is one of the most commonly used glucocorticoids. It has been shown to reduce the development and progression of erosion. However, it is still not known whether the potential benefits are maintained after treatment or if they are outweighed by long-term side effects.[2,4,5,96-98]

Steroids are still not the therapy of choice in early disease. Their use depends on the severity of the disease. They are used for patients refractory to other treatments and for those who have low tolerance for NSAIDs. They are also used to induce remission in severe RA and maintain it until the delayed response of an eventual disease-modifying drug therapy started at the same time. Side effects for prednisolone have been described such as growth retardation in children, bone loss and osteoporosis.[2,4,5]

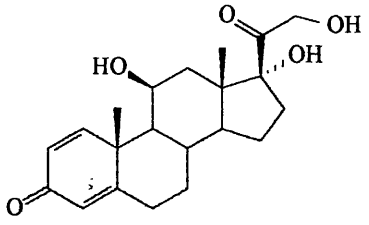


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Figure 17: Structure of prednisolone.

Although other steroids are being developed to decrease those side effects, the potency of prednisolone still makes it the substance of choice for anti-inflammatory treatment in RA. If prednisolone could be delivered as an inactive prodrug to the site of inflammation and released specifically there, it is believed that the side effects due to this drug would be drastically reduced, making it a very powerful and efficient therapy for RA. These aspects motivated the choice of prednisolone as the second drug moiety for the present study.

5. AIM AND OBJECTIVES

5.1 AIM

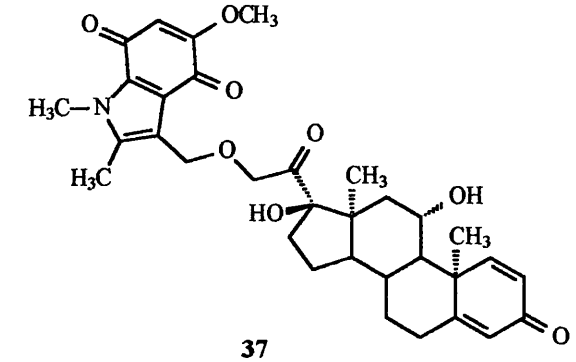
MMC analogues exhibit an optimum "trapping" potential when hypoxia is profound and imidazole analogues are selectively retained in hypoxic tissue by reductive metabolism. This is believed to form the basis for their selectivity. Consequently, the idea is to use a bioreductive compound or carrier for the specific targeting of a drug to areas of hypoxic and/or ischaemic tissue, in which the desired drug species is linked to a non-cytotoxic counterpart.

The method proposed would involve the synthesis of prodrugs capable of targeting drugs to sites of inflammation within the body associated with hypoxia and/or ischaemia, *e.g.* to the synovium in the treatment of rheumatoid arthritis. This method not only has the effect of reducing the risk of systemic side effects of the drug but also enhances the therapeutic effect of the drug [99-101]. The bioreductive prodrugs have to be substantially stable in an oxygenated environment, but under hypoxic conditions, reductive activation occurs. The therapeutic agent is then released from the bioreductive moiety.

Some redox drugs have already been used in the past to act as trigger moieties for drug delivery systems. However, after delivery of the drug, that is to say after activation of the prodrug, the bioreductive moiety is often converted to a cytotoxic species[80-83,102,103]. Providing a nucleophilic centre within the bioreductive compound itself may reduce the cytotoxicity of the bioreductive moiety. Following release of the drug, an alkylating centre is formed. However, the proximity of a nucleophilic centre would ensure that intramolecular alkylation occurs in preference to alkylation of any biomolecules such as DNA. In this way, substantially no cytotoxic species would be formed. Such systems are said to be "self-alkylating". Alternatively, the bioreductive compound may be rendered non-cytotoxic following drug delivery by means of the introduction of steric hindrance preventing nucleophilic attack at the newly formed reactive centre. This is achieved by the presence of a bulky group either at or in close proximity to any potential alkylating centre generated after drug release.

5.2 *OBJECTIVES*

In the present study, it was proposed to synthesise two series of agents with potential as prodrugs; a first series being based on an indoledione trigger, the second on a 5-nitro-2-thienylmethyl trigger. The drugs moieties selected were prednisolone, isoquinolinone and its 5-iodo and 5-bromo analogues, and aspirin.



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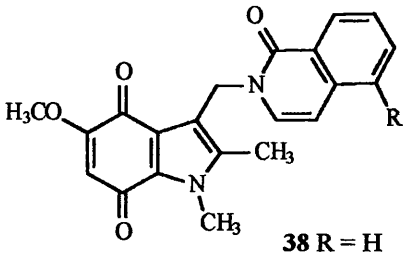
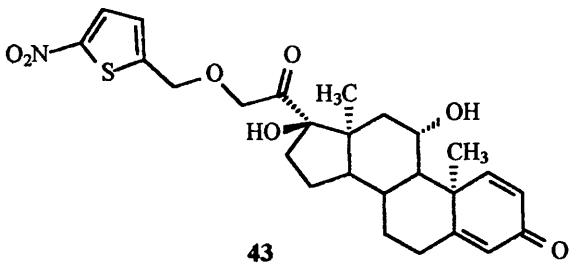


38 R = H
39 R= I
40 R= Br
41 R= NH_2



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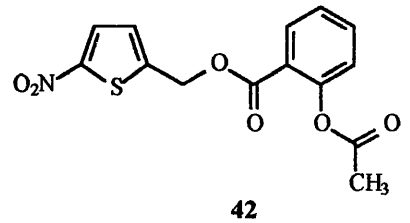

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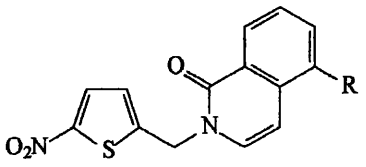

44 R = H
45 R = NH_2
46 R = I
47 R = Br

Figure 18: Proposed prodrugs with an indoledione trigger and prednisolone or isoquinolin-1-one effectors and with a 5-nitro-2-thienylmethyl trigger and prednisolone, isoquinolin-1-one or aspirin effectors.

The two series of prodrugs were designed to be tested for drug release under reductive conditions. Chemical reductive systems were to be investigated to bring evidence for drug release after the chemical reduction of the paraquinone and of the nitro group, motifs which are known to be bioreduced *in vivo*.[24-27] The potential drug release was proposed to be monitored by HPLC and proton NMR spectroscopy.

RESULTS AND DISCUSSION

6. 1,2-DIMETHYL3-(HYDROXYMETHYL)-5-METHOXY-INDOLE-4,7-DIONE TRIGGER.

The first synthetic target was 21-O-(1,2-dimethyl-4,7-dioxo-5-methoxyindol-3-ylmethyl)prednisolone (**37**). Composed of an indole-4,7-dione trigger, namely 1,2-dimethyl-3-(hydroxymethyl)-5-methoxyindole-4,7-dione (**11**), and of prednisolone, the retrosynthetic analysis was, at first, fairly simple and convergent. The two subunits could be treated separately and linked in the final stage of the synthetic pathway. The drug moiety was commercially available and the trigger chosen, for the first part of the present study, was a known substance for which chemical routes had been previously designed[50,52]. Prednisolone could be linked to the trigger parent compound by nucleophilic substitution at the chloromethyl group attached at position 3 of the indole nucleus.

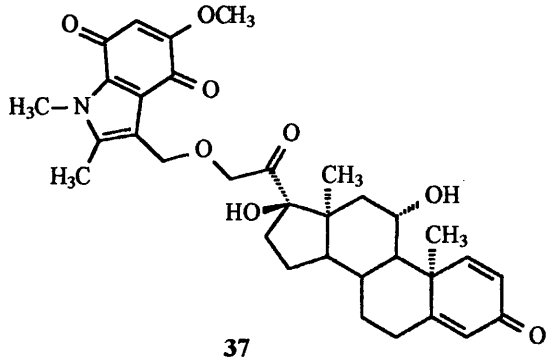

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Figure 19: Structure of the first synthetic target compound **37**.

The synthesis of the trigger, although previously reported[51], proved to be more complicated than was expected. The instability and poor reactivity of some intermediates decreased the reported yields significantly[51]. Investigation of other existing routes was required[50]. The unreactivity of prednisolone with the indole trigger in basic media required a change in strategy and the introduction of a linker.

The synthesis of EO9 analogue indolediones has been investigated in the past, but recent studies have outlined some new chemical pathways. Naylor *et al* [50,51] proposed a first chemical route to 1,2-dimethyl-3-(hydroxymethyl)-5-methoxyindole-4,7-dione (**Figure 20**)[50], soon complemented by an updated method, leading to the same target in an improved overall yield.[51]

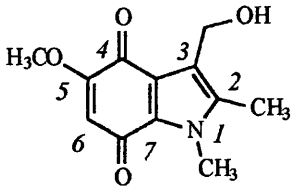

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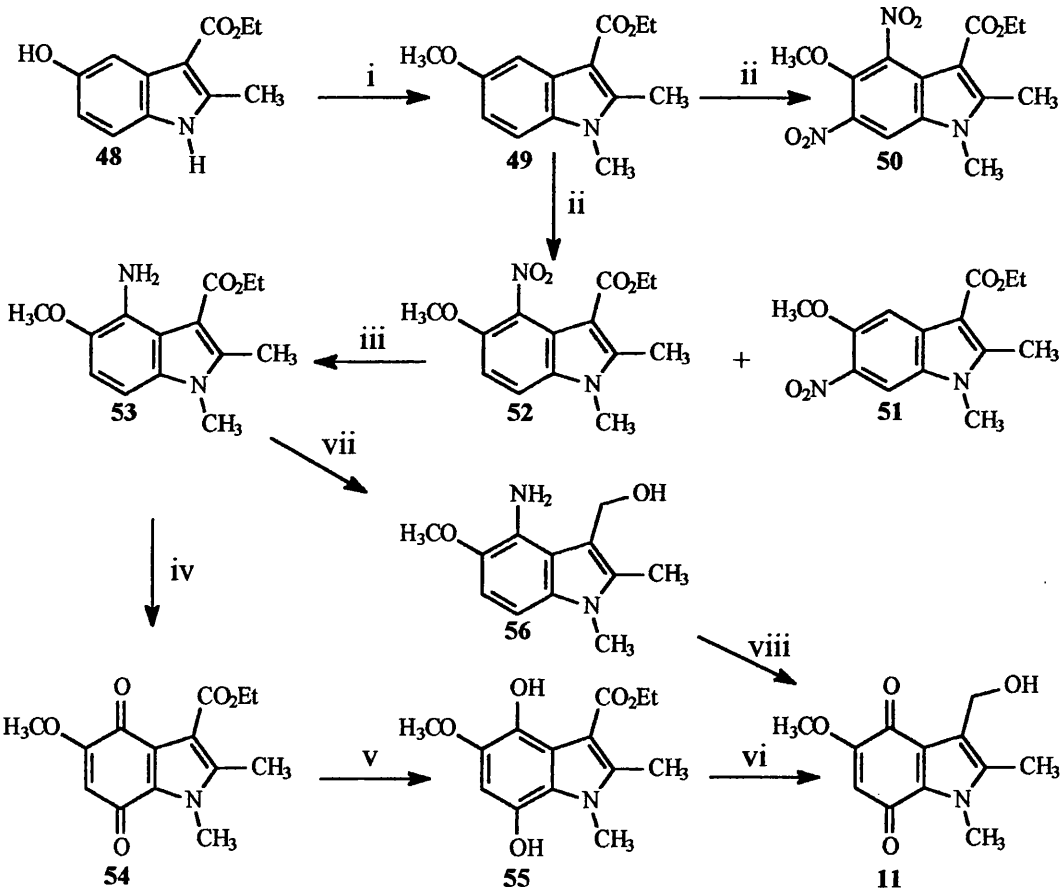
Figure 20: Structure of 1,2-dimethyl-3-(hydroxymethyl)-5-methoxyindole-4,7-dione.

As discussed in part **3.3**, previous studies [50-52,57] have highlighted the mechanistic importance of indole-4,7-dione-based triggers. Upon reductive activation, 3-indolyl carbinyl in derivatives of **11** potentially undergo a "retro-Michael" elimination (**Scheme 3**) generating a potential alkylating agent.

However, substituents on indoledione-based analogues of EO9 are shown to have a considerable importance on the redox potential, inducing or suppressing hypoxia selectivity, influencing cytotoxicity, substrate selectivity for the activating enzymes *etc.* According to the literature[57], **11** would be characterised by a reduced potential toxicity due to the influence of the methoxy group at position 5 and the steric hindrance induced by the methyl group at position 2, and increased hypoxia selectivity from the influence of the hydroxymethyl substituent at position 3. Consequently, **11** showed a high potential for the release of drugs in a reductive environment together with a reduced risk of genotoxicity.[57]

6.1 *PREPARATION OF 1,2-DIMETHYL-3-(HYDROXYMETHYL)-5-METHOXY-INDOLE-4,7-DIONE (11)*

There are a number of ways to synthesise compound **11**. Naylor *et al* have developed three routes[50,51]. The choice of the method was based on the high yields published by Naylor *et al*[51] in their latest chemical route to **11**[51]. However, some modifications proved to be necessary (**Scheme 11**).



Scheme 11: Synthetic pathway 1 to 1,2-dimethyl-3-(hydroxymethyl)-5-methoxy-indole-4,7-dione **11**. Reagents: i, KH, MeI, DMF; ii, HNO_3, AcOH; iii, Sn, HCl, H_2O, EtOH; iv, $(KO_3S)_2NO$, Me_2CO, NaH_2PO_4/Na_2HPO_4/pH 6.0; v, $Na_2S_2O_4$; vi, DIBAL-H then $FeCl_3$; vii, $LiAlH_4$, THF; viii, $(KO_3S)_2NO$, Me_2CO, NaH_2PO_4/Na_2HPO_4/pH 6.0.[45]

The starting material, ethyl 5-hydroxy-2-methylindole-3-carboxylate **48**, was commercially available. Deprotonation and dimethylation using iodomethane afforded **49** in 85% yield in the first step. Nitration of **49** using concentrated nitric acid in acetic

acid gave a mixture of regioisomers **51** and **52** in 14% and 63% yield respectively. Increasing the reaction time gave the dinitro compound **50** in 80% yield. It is known [104] that indoles possessing electron-donating groups at position 1 and 2 and a strong electron-withdrawing group at position 3 (carbonyl) usually direct nitration at position 6 and eventually 4 in a lower yield. Substitution at position 3 of the pyrrole ring protects the benzene ring against oxidative attack and allows nitration to take place. The presence of the methoxy group at position 5 directs nitration to position 4 preferentially. The 4-nitro group is not sufficiently deactivating (as an electron-withdrawing group) to prevent a second nitration (at position 6) at longer reaction times, affording **50**.[104]

Reduction of **52** with tin powder in conc. aq. HCl gave the aminoindole **53**. This compound proved to be unstable, probably due to the presence of three relatively strong electron-donating groups. It was therefore used directly in the next step without further purification. After optimisation of the pH of the buffer required, compound **53** was oxidised with Fremy's salt to the paraquinone derivative **54**. This method of oxidation, called the Teuber reaction, allows the oxidation of phenols to the corresponding quinone. Aromatic amines get similarly oxidised but the product formed depends on the structure of the starting amine: primary aromatic amines often undergo an oxidative condensation, secondary aromatic amines are oxidised to the quinone imines which are then hydrolysed to quinones. The transformation proceeds *via* a radical mechanism. [105]

The final step towards **11** was based on three consecutive reactions, first reduction of the quinone to the hydroquinone derivative using sodium dithionite, followed by the reduction of the ester with diisobutylaluminium hydride and work-up under air to ensure re-oxidation of the hydroquinone to the quinone **11**. These three combined steps proved to be unreliable and led to the expected target on only one occasion, with a yield of 6.4%. The air-sensitivity of the intermediate hydroquinone-ester was postulated to be critical but no improvement was observed when the first two steps were carried out completely under inert atmosphere.

Efforts were then concentrated on the reduction of the ester using an alternative method. One role of the ester in the synthetic route was to increase the stability of the

aromatic amine by withdrawing electron-density from the aromatic system and, possibly, by intramolecular hydrogen bonding; therefore, the reduction step was introduced after formation of the quinone. In the method used by Naylor *et al*[51], the quinone was reduced to the hydroquinone before the ester was reduced. Hydrogen bonding between the 4-hydroxyl group on the hydroquinone and the oxygen of the carbonyl from the ester functional group was predicted to facilitate the reduction of the ester. Analogous reductions with sodium borohydride and with lithium aluminium hydride also failed to give the hydroxymethyl derivative, under a variety of conditions.

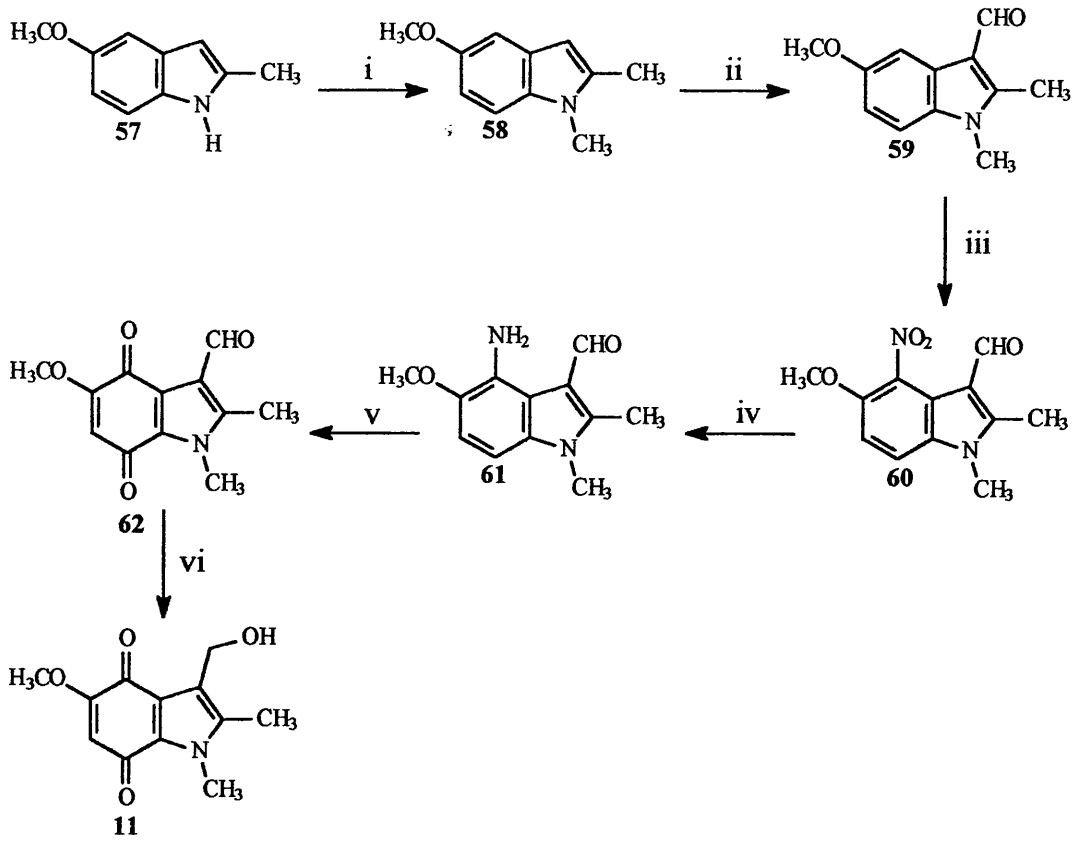
Since reduction of the ester had been unsuccessful, attempts were then made to hydrolyse or dealkylate the ester and subsequently reduce the carboxylic acid. The ester **54** was resistant to all attempts at hydrolysis under basic or acidic conditions.

Similarly, attempted dealkylations using chlorotrimethylsilane and sodium iodide in acetonitrile[106], and aluminium bromide and tetrahydrothiophene[107] also proved to be unsuccessful. It is reported that ethyl esters are amongst the most difficult esters to dealkylate[106,107]. However, although very strong conditions were used, no alteration of the molecule was observed.

The oxidation state of the substituent at position 3 was then considered. Its alteration for a group with a lower oxidation state but remaining a stabiliser of the amine would be an option to overcome the unreactivity of the ester.

The initial route to **11** designed by Naylor *et al*[50] (**Scheme 12**) was similar to the one discussed above[51] (**Scheme 11**) in the general strategy of building up the indoledione-based trigger. In this second route, the starting indole nucleus was only substituted at positions 2 and 5, and the one-carbon electron-withdrawing group at position 3 was introduced in the second step in the form of an aldehyde (lower oxidation state). In comparison to the route described above, the aldehyde derivative obtained after quinone formation was expected to be easier to reduce.

The yields of the intermediate steps were lower than for the previous method used but led to the trigger **11**.



Scheme 12: Second synthetic pathway to 1,2-dimethyl-3-(hydroxymethyl)-5-methoxy-indole-4,7-dione introducing a group with a lower oxidation level at position 3. Reagents: i, NaH, MeI, DMF; ii, DMF, $POCl_3$ then NaOAc, H_2O; iii, fuming HNO_3, AcOH; iv, Sn, HCl, H_2O, EtOH; v, $(KO_3S)_2NO$, Me_2CO, NaH_2PO_4/Na_2HPO_4/pH 6.0; vi, $NaBH_4$, MeOH, Ar then air.[44]

N-Methylation of the commercially available compound **57** in basic conditions in the presence of iodomethane gave **58** in high yield. Formylation of **58** was initially carried out by using phosphoryl chloride and N-methylformanilide. The Vilsmeier complex had to be formed before addition of the starting material and the yield obtained was low. The alternative one-pot reaction using DMF and phosphoryl chloride allowed the formation of the complex *in situ*, and gave a 10% yield improvement without increase in reaction time. Position 3 of the indole ring is known to be the most reactive towards electrophilic attack[104]. Substituents at this position also influence the reactivity of the fused benzene ring by influencing the electron density[104]. The aldehyde was identified as being a stabilising factor for the amine derivative but also as a directing factor for the preceding nitration reaction[104].

The nitration of 1,2-dimethyl-5-methoxyindole-3-carboxaldehyde was carried out in fuming nitric acid. Nitration was mostly directed at position 4 (60% yield) although the regioisomer was detected in negligible amount. Reduction of **60** to the amine, followed by oxidation to the paraquinone, was achieved *via* the method previously described. The aldehyde of **62** was reduced with sodium borohydride to afford 1,2-dimethyl-3-hydroxymethyl-5-methoxyindole-4,7-dione **11** in 33% yield, with much starting material recovered.

The proton NMR data matched the structure of the compound. The signal for H-6 was observed as a singlet at δ 5.61 followed by four singlets in the aliphatic region assigned, respectively, to the methylene protons of the 3-hydroxymethyl group at δ 4.65, the methoxy protons at δ 3.86, the N-methyl protons at δ 3.81 and the 2-methyl protons at δ 2.21.

This linear multi-step sequence gave **11** in low overall yield and was expensive in time and reagents. The small amounts that could be obtained limited the subsequent coupling reactions using **11**.

In their improved route (**Scheme 11**), Naylor *et al* [51] highlighted the possibility of reducing the ester in the 4-aminoindole **53**, *before* the oxidation to the paraquinone. This sequence generated an intermediate which presented no stabilising group for the intermediate aromatic amine. However, investigation of this last route gave surprising results. Reduction of ester **53**, using lithium aluminium hydride, afforded 4-amino-1,2-dimethyl-3-(hydroxymethyl)-5-methoxyindole (**56**) in high yield. Interestingly, no signs of autoxidation of the amine were observed during the work-up but the intermediate **56** was used in the next step without further purification. Oxidation using Fremy's salt gave **11** in 75% yield[105].

6.2 *POTENTIAL PRODRUGS DERIVED FROM 1,2-DIMETHYL-3-(HYDROXYMETHYL)-5-METHOXY-INDOLE-4,7-DIONE AND PREDNISOLONE*

The hydroxymethylindoledione **11** was the starting point of a wide variety of analogues used for studies on hypoxia selectivity and cytotoxicity[50,51]. Many of those were different from **11** by the nature of their substituent at position 3, implying a reasonable ease of reaction at this position of the molecule.

The first approach was to attach prednisolone to an electrophilic derivative of 1,2-dimethyl-3-hydroxymethyl-5-methoxyindole-4,7-dione bearing a leaving group at the 3-CH_2, *via* a nucleophilic substitution. 3-(Chloromethyl)-1,2-dimethyl-5-methoxy-indole-4,7-dione **63** was chosen as the derivative of **11** to react with prednisolone in a basic medium. It was generated by treatment of **11** with thionyl chloride.

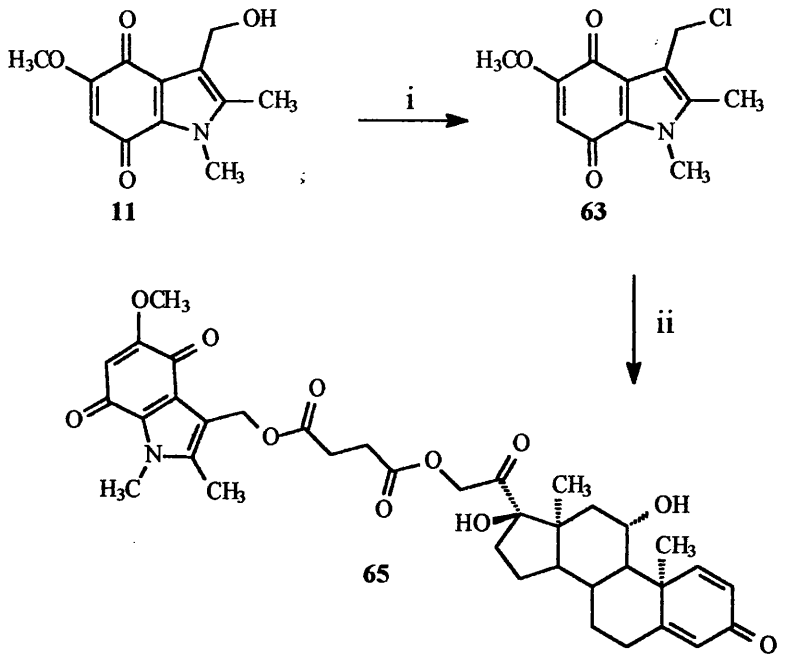
Sodium hydride was used to deprotonate prednisolone to generate the potentially nucleophilic 21-alkoxide species. Treatment of this alkoxide with **63** failed to give the expected conjugate **37**, even in the presence of the nucleophilic catalyst, sodium iodide at various temperatures. Replacement of the base with potassium hydride, which was easier to handle, also failed to bring about the desired nucleophilic substitution. Similar experiments using stronger bases (lithium bis(trimethylsilyl)-amide and n-butyl lithium) under various conditions of time and temperature also failed. Since all of these bases are strong and will easily generate the alkoxide, it is possible either that the alkoxide is not sufficiently nucleophilic to react with the relatively weak electrophile in **63** or that the nucleophile is relatively hard and the electrophile is too soft.

An alternative target in which the prednisolone is linked to the indoledione through a carbonate was then investigated. Compound **11** decomposed on treatment with phosgene, rather than generating the corresponding chloroformate. Synthesis of the 4-nitrophenyl carbonate **64** of **11** was achieved by treating **11** with 4-nitrophenyl chloroformate under basic conditions. Treatment of this carbonate with the alkoxide of prednisolone under the conditions previously described resulted in decomposition of the electrophile.

The poor reactivity was suspected to be the consequence of a lack of interaction between nucleophile and electrophile due to steric hindrance. The introduction of a linker under the form of a longer carbon chain was then considered. The commercially available prednisolone-21-hemisuccinate was selected. The hemisuccinate derivative is metabolised *in vivo* by esterases to prednisolone[102], thus not altering the activity of the drug.

Denny *et al* [101], in the context of the design of anticancer prodrugs for use in ADEPT and GDEPT (antibody-directed and gene-directed enzyme-prodrug therapies respectively), described the three elements that should compose a suitable prodrug: trigger-linker-effector. Each unit has to be clearly distinct and has a specific role. The trigger unit is characterised by its tumour selectivity and its selective activation by the appropriate enzyme, the released effector has to be potent and diffusible, the linker has to be a separate and distinct part of the prodrug or just a mechanism by which activation of the trigger leads in changes in the effector group. Although the present work is not included in ADEPT or GDEPT therapies, the prodrug design approach applies to the present study. The hemisuccinate moiety in this particular case matches the definition of the linker unit given by Denny *et al* [101].

To limit the number of influencing factors on the reaction, the sodium salt of prednisolone-21-hemisuccinate was used under basic conditions and reacted with **63** under reflux (**Scheme 13**) to afford conjugate **65** in reasonable yield. Evidence for the formation of the conjugate was obtained by ^{1}H NMR. Shifting of the indole 3-CH_2 group signal from δ 4.86 to δ 5.16 was observed due to the more deshielding effect of the ester, compared to chlorine in the precursor. In $CDCl_3$ and in DMSO, this CH_2 resonated as a singlet. In contrast, the spectrum run in CD_3OD showed that the two methylene protons are diastereotopic (magnetically inequivalent), demonstrating that the indoledione unit was attached to a chiral molecule. Mass spectrometry and accurate mass determination confirmed the presence of the conjugate.



Scheme 13: Synthetic pathway to 1-[(1,2-dimethyl-4,7-dioxo-5-methoxyindol-3-yl)methyl]-4-(prednisolon-21-yl) butanedioate. Reagents: i, $SOCl_2$; ii, prednisolone-21-hemisuccinate sodium salt, THF, heat.

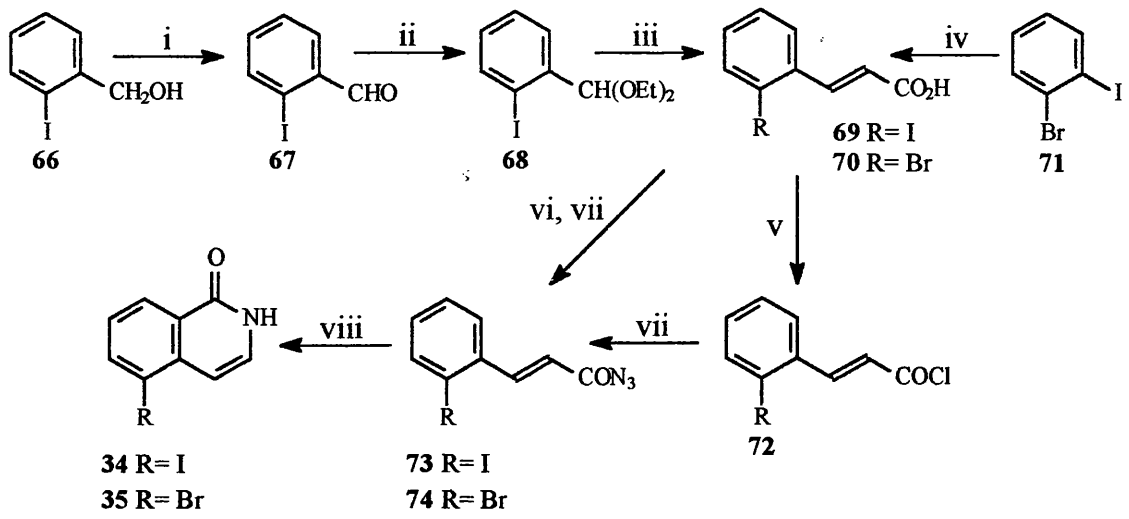
Carboxylates are softer nucleophiles than alkoxides. Using the softer carboxylate in prednisolone 21-hemisuccinate salt as the nucleophile, the synthesis of the first target **65** was achieved, linking a compound, which can be bioreductively activated, to a steroidal drug moiety. The ester linkages present in the molecule constituted an interesting element to observe during the release studies.

6.3 *POTENTIAL PRODRUGS DERIVED FROM 1,2-DIMETHYL-3-(HYDROXYMETHYL)-5-METHOXY-INDOLE-4,7-DIONE AND ISOQUINOLIN-1-ONES / MELAMINES*

6.3.1 *Isoquinolin-1-ones.*

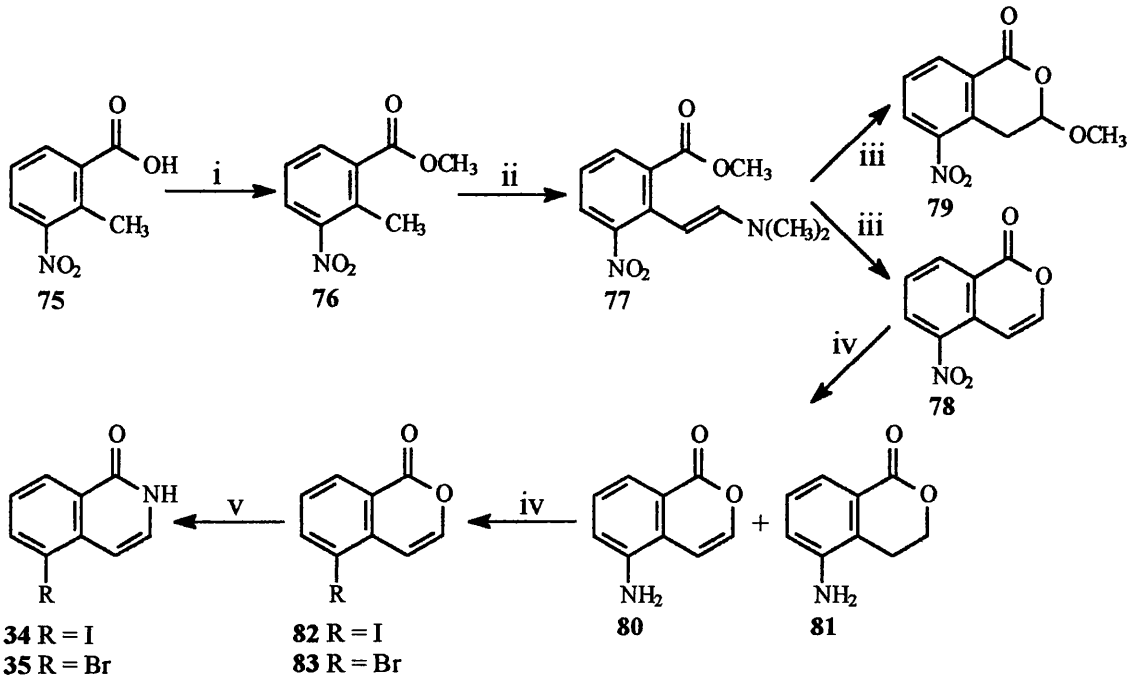
5-Substituted isoquinolinones have been reported[83,92-94,109,110] to be potent PARP inhibitors. PARP is an enzyme activated by DNA damage and catalyses its repair. Reactive oxygen species have been reported to be important inducers of PARP. As previously mentioned in chapter **1**, part **1.5**, and in chapter **2**, the rheumatic state is characterised by a redox imbalance, hypoxia / reperfusion sequences and hyperactive immunity that trigger tissue damage, NO production and ROS generation leading to increased levels of PARP.[83,92-94,109,110] It is believed that inhibition of PARP could result in an important reduction of the inflammation in the case of rheumatic joints.[89]

Several different routes to 5-substituted isoquinolin-1-ones have been described. For example, 5-nitroisoquinolin-1-one was formed by rearrangement of 5-nitroisoquinolin-N-oxide[111]. Berry *et al*[83] and Eloy and Deryckere[112] have used the Curtius rearrangement of 2'-substituted cinnamic acids, followed by cyclisation of the 2-arylethenylisocyanates at high temperatures (**Scheme 14**). This cyclisation is restricted to synthesis of isoquinolinones where the 5-substituent is not strongly electron-withdrawing and is thermally stable. In the routes developed by Berry *et al*[83], 2'-bromocinnamic acid **70** (R = Br) was synthesised in an iodine-selective Heck coupling of propenoic acid with 2-bromoiodobenzene **71**, whereas 2'-iodocinnamic acid **69** (R = I) was prepared by Knoevenagel condensation of 2-iodobenzaldehyde **67** (from oxidation of 2-iodobenzyl alcohol **66**) with propanedioic acid. The iodocinnamic acid was converted into acid chloride; treatment with sodium azide gave the acid azide **72**. Curtius rearrangement of the acid azide at high temperature led to the cyclisation. The resulting 5-iodoisoquinolinone **34** was reported to be obtained in 42% yield; the 5-bromo analogue **35** was obtained with a 10% yield. However, these sequences have proved to be somewhat unreliable and, since high temperatures and potentially explosive acyl azide intermediates are involved, an alternative route to the 5-haloisoquinolinones was sought.



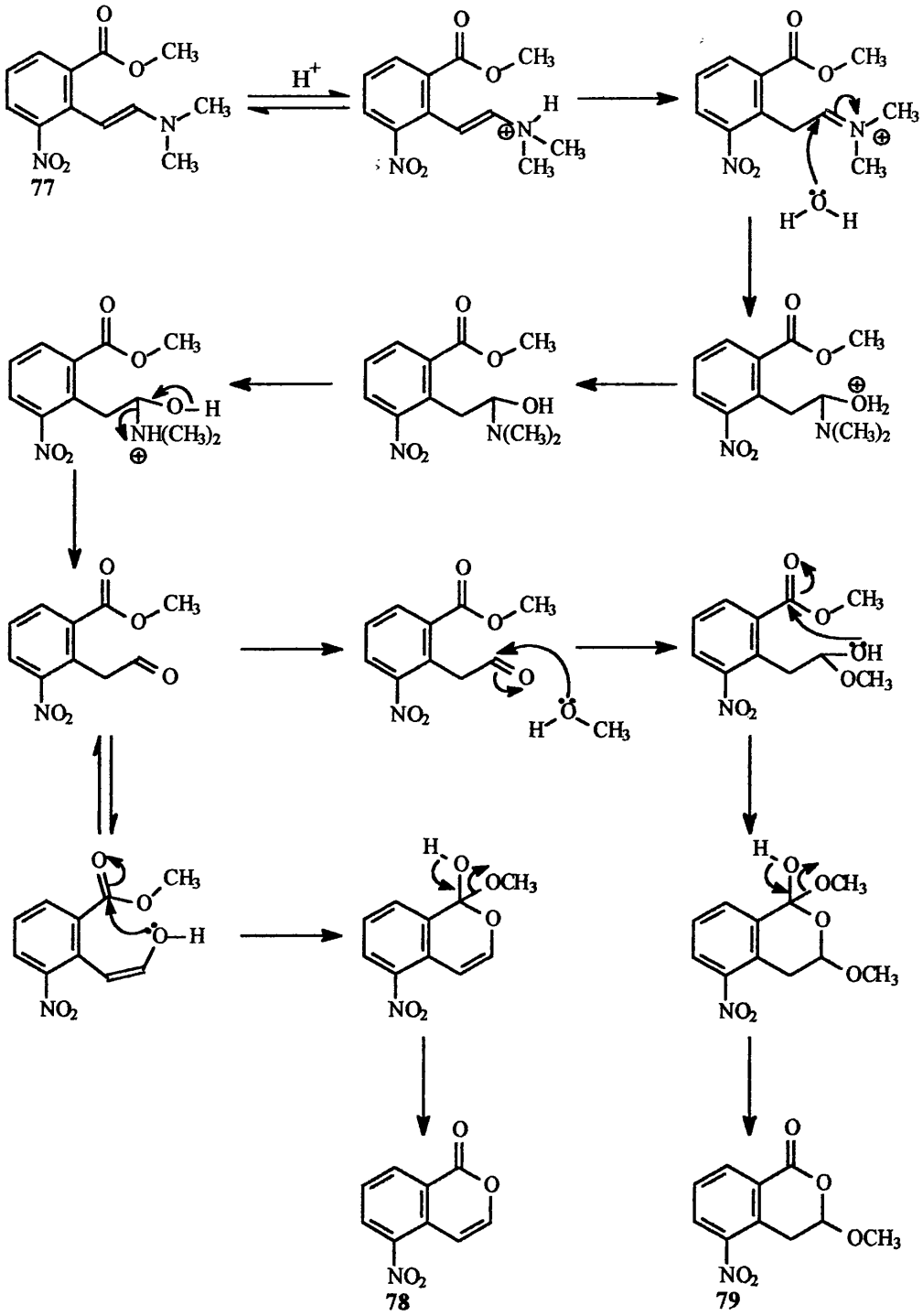
Scheme 14: Synthesis of isoquinolinones. Reagents: i, pyridinium dichromate, CH_2Cl_2; ii, $HC(OEt)_3$, $SOCl_2$, EtOH; iii, $CH_2(CO_2H)_2$, piperidine, pyridine; iv, $H_2C{=}CHCO_2H$, $Pd(OAc)_2$, Et_3N, EtCN; v, $SOCl_2$, DMF; vi, EtO_2CCl, Et_3N, Me_2CO; vii, NaN_3, water, 1,4-dioxane or Me_2CO; viii, heat, Ph_2O or $(MeOCH_2CH_2OCH_2CH_2)_2O$.

Parveen[113] designed another route to the 5-haloisoquinolin-1-ones from 5-aminoisocoumarin[114] (**Scheme 15**), which was employed and optimised in the present work.



Scheme 15: Synthesis of 5-bromo and 5-iodoisoquinolinone. Reagent: i, MeOH, $SOCl_2$; ii, DMF, DMFDMA; iii, SiO_2; iv, THF, aq. HCl, Pd/C, H_2; v, H_2O, $NaNO_2$, aq. H_2SO_4, CuBr/KBr or KI; vi, 2-methoxyethanol, NH_3.

2-Methyl-3-nitrobenzoic acid **75** was converted into its methyl ester **76**. Condensation with dimethylformamide dimethylacetal (DMFDMA) in DMF at high temperature led to compound **77**, as an unpurified intermediate. The ^{1}H NMR spectrum of crude **77** shows the alkenic protons at δ 6.28 and δ 5.60 as two doublets with a coupling constant of 13.5 Hz, suggesting that it is the *E*-stereoisomer (*trans* configuration). Cyclisation of the enamine **77** on silica gel gave 5-nitroisocoumarin **78** in 32% overall yield. The cyclisation appears to be catalysed by the acidity of the silica gel (**Scheme 16**). ^{1}H NMR spectroscopy confirmed the compound formation with no sign of the $N(CH_3)_2$ or of the methyl ester peak. The 4-H resonated as a doublet at δ 7.39 coupled to 3-H, also as a doublet at δ 7.44. 8-H appeared at δ 8.64 as a doublet of doublets, a consequence of the *ortho* coupling to 7-H and *meta* coupling to 6-H. The same splitting pattern was observed for 6-H at δ 8.51. 7-H resonated as a *pseudo* triplet due to the neighbouring 6-H and 8-H.



Scheme 16: Proposed mechanism for the cyclisation of the enamine and its side product.

Further elution gave 3-methoxy-5-nitro-3,4-dihydroisocoumarin **79** in 10% yield. The ^{1}H NMR spectrum shows aromatic peaks slightly downfield than those of **78**, possibly due to the lack of an extended conjugated system. The methoxy group is seen as a singlet at δ 3.55, the adjacent methine proton resonates as a triplet at δ 3.59 due to vicinal coupling (coupling constant J = 3.2 Hz) with the methylene protons themselves coming as a doublet at δ 5.52. The splitting pattern observed for the methylene protons is unexpected; they are adjacent to a chiral centre, therefore the two protons are expected to be magnetically inequivalent and two signals should be observed. The reasons for this are still unclear.

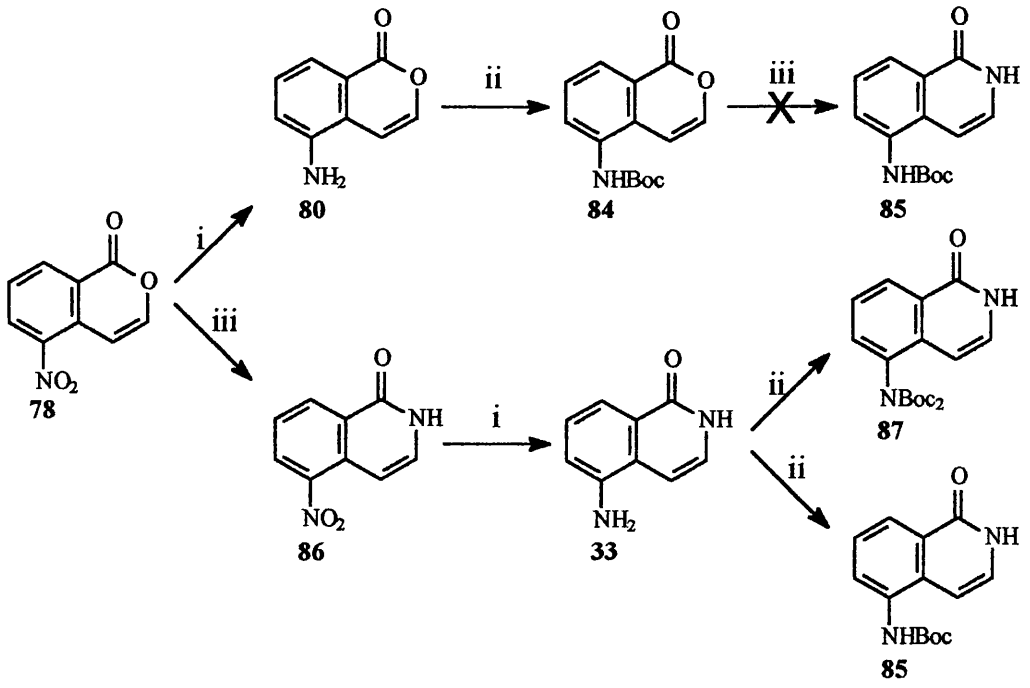
5-Nitroisocoumarin **78** was converted into 5-aminoisocoumarin **80** by catalytic hydrogenation (**Scheme 15**). Reduction of the double bond of the isocoumarin ring occurred with prolonged reaction time giving 5-amino-3,4-dihydroisocoumarin **81**. After one hour under the described conditions, a mixture of 50% 5-amino-3,4-dihydroisocoumarin **81** / 50% 5-aminoisocoumarin **80** was obtained. Tin(II) chloride has been reported by Bellamy and Ou[115] as an alternative reducing agent, limiting the risk of over-reduction. Tin(II) chloride reduces selectively the aromatic nitro group and does not affect the double bond but the yield and the reaction time are less satisfactory. Evidence for the formation of **80** was obtained by ^{1}H NMR. The NH_2 signal was seen at δ 3.96 as a broad singlet and the aromatic protons are shifted upfield, typical of the shielding effect of the amino group compared to the nitro group (6-H shifts from δ 8.51 to δ 7.04). The over-reduced compound **81** showed 3-H_2 and 4-H_2 as triplets at δ 4.5 and δ 2.8, respectively.

Diazotisation of 5-aminoisocoumarin with sulfuric acid and sodium nitrite, and treatment with copper(I) bromide / potassium bromide afforded 5-bromoisocoumarin **83** in 37% yield (**Scheme 15**). The iodo analogue **82** was obtained similarly from diazotisation of 5-aminoisocoumarin in concentrated hydrochloric acid and sodium nitrite, followed by treatment with potassium iodide. Reaction between the sodium nitrite and the sulfuric acid generates nitrous acid converting the aromatic amine into the diazonium salt. The resulting diazonium species is very reactive and can be displaced by a nucleophile (Sandmeyer reaction). The mechanism was long thought to be S_N1 but the hypothesis of a radical mechanism has also been reported.[116] In both

cases, the absence of the broad NH_2 peak on the ^1H NMR and the shifting downfield of the aromatic protons due to the negative inductive effect from the bromide or iodide matched the compound's structure.

Finally, ammonia in boiling 2-methoxyethanol gave the expected compounds, 5-iodo and 5-bromoisoquinolin-1-one **34** and **35** in 68% and 71% yields, respectively. TLC analysis and the observation of a broad single peak at δ 11.1 ppm (highly characteristic of the hydrogen-bonded NH of isoquinolin-1-ones) in the ^1H NMR spectrum indicated the formation of the compounds.

5-Aminoisoquinolinone **33** was recently reported to be a powerful inhibitor of PARP. McDonald *et al* [94] reported the route to 5-aminoisoquinolinone hydrochloride from 5-nitroisocoumarin, in other words using the same first steps as Parveen[113]. Bearing in mind that 5-aminoisoquinolinone was to be attached to a trigger, it has to be part of the strategy to protect the amino group at position 5.



Scheme 17: Proposed chemical pathways to the Boc-protected 5-aminoisoquinolin-1-one. Reagent: i, Pd/C, HCl, THF, H_2; ii, Boc_2O, Et_3N, DCM, DMF; iii, 2-methoxyethanol, NH_3.

Parveen's route to the 5-aminoisocoumarin[113] **80** was initially examined. Protection of the 5-amino group with a Boc group and treatment of the resulting 5-Boc-amino-isocoumarin **84** with ammonia in 2-methoxyethanol would have led to **85** (**Scheme 17**). The ^{1}H NMR spectrum obtained of **84**, after protection of the amine **80**, showed the 9 protons of the Boc protecting group as a singlet at δ 1.54.

Treatment of **84** with ammonia gave an unexpected result. TLC analysis brought evidence for the complete conversion of the starting material to a new compound. The indicated polarity of the substance seemed, however, too low compared to the expected polarity of **85**. ^{1}H NMR results suggested that the compound formed was not the expected isoquinolin-1-one **85** but may be a derivative of **84** produced by ring opening by methoxyethanol and rearrangement. However, the structure of this compound has not been completely elucidated.

The reaction was repeated and gave the same unique compound. Solvent effects were investigated. 2-Methoxyethanol was suspected to be at the origin of the ring opening at position 1 of the isocoumarin. A high-boiling non-nucleophilic solvent, 1,2-diethoxyethane, was used instead and the same compound was formed. Replacement of the oxygen by nitrogen was not achieved with this method.

A different strategy was then chosen. The replacement of the oxygen of the 5-nitro-isocoumarin **78** by nitrogen to obtain the isoquinolinone **86** was carried out before the reduction of the nitro group and subsequent Boc protection (**Scheme 17**). The 5-nitroisoquinolin-1-one **86** was obtained in 60% yield. The NH group was identified by ^{1}H NMR spectrum at δ 11.8, confirming, together with mass spectrometric results, the formation of the compound.

Compound **86** was then reduced to the amine **33** following the procedure previously described. Shifting of the aromatic protons upfield was observed, as for compound **80,** under the electron-donating effect of the NH_2 group. The exocyclic amine of **33** was then protected by treatment with di-*tert*-butyl dicarbonate. The Boc compound **85** was obtained in 60% yield, despite the very poor solubility of the starting material in

suitable reaction media. The diBoc derivative **87** was also obtained in small yields from some runs.

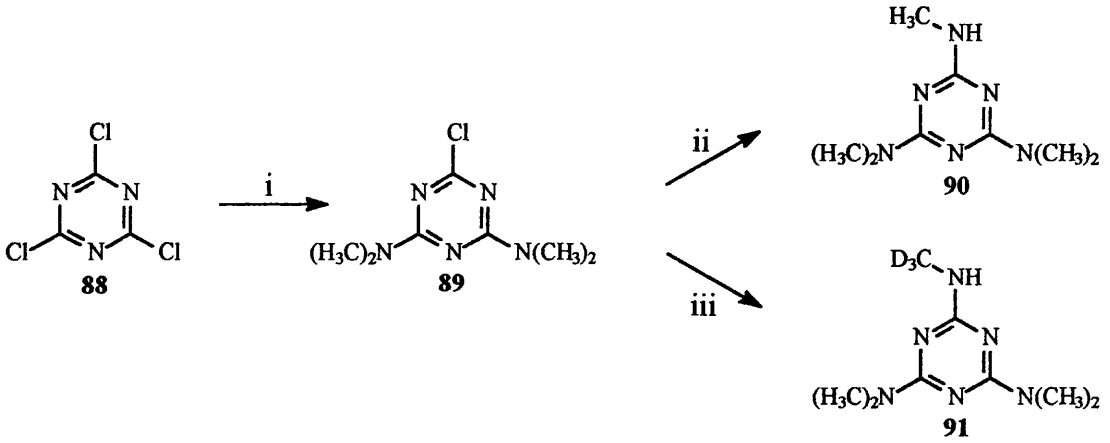
6.3.2 *Pentamethylmelamine*

In the present work, pentamethylmelamine was used as a model drug moiety, since it was relatively easy and cheap to synthesise, compared to isoquinolin-1-ones. One of the reasons that motivated the use and study of pentamethylmelamine and its analogue hexamethylmelamine in the past was their antitumour properties. They proved to be neurotoxic and were not used for that therapeutic purpose. Other uses have been recorded for those substances but the chemical features of pentamethylmelamine were the only interest for this study. [117,118]

Pearlman and Banks[119] described the synthesis of a series of 2,4-bis-substituted amino-6-chloro-1,3,5-triazines. Cyanuric chloride **88** was used as the starting material for the preparation of the substituted chlorodiaminotriazines. The preparation of un-symmetrically substituted compounds involved following a strategy relying on the order of introduction of the different amines and their relative reactivity.[119] Reaction of cyanuric chloride with ammonia replaced one chlorine substituent below 0°C and two below 100°C, interaction with dimethylamine replaced all three chlorine atoms at 25°C.[119] The basicity of the amine was proved to be only one of the factors influencing the reactions. Sufficiently low temperature allowed, even in the case of reactive amines, displacement of only one chlorine.

In this case, it was desired to introduce only one kind of amine, namely dimethylamine, to replace two of the three chlorines in the first step but its reactivity required careful temperature control to avoid displacement of the third chlorine on the triazine. Cyanuric chloride **88** was treated with dimethylamine in aqueous medium at 0°C. Heating the mixture to 20°C allowed the displacement of the second chlorine to give **89** in good yield. The symmetry of the molecule is responsible for the presence of one singlet for the twelve protons of the two $N(CH_3)_2$ groups in **89** at δ 3.13 in the proton NMR spectrum.

Borkovec and DeMilo[120] reported a method for the displacement of the last chlorine by an amine of a different nature from the ones already present on the ring to form an asymmetrically substituted triazine. It proceeded under harsher conditions compared to the previous reaction conditions. In the present work, displacement of the last chlorine atom was achieved by treating **89** with methylamine under boiling concentrated basic aqueous conditions to give **90** in good overall yield (**Scheme 18**). Evidence for the formation of **90** was obtained by ^{1}H NMR. The expected isolated methyl peak was observed as a singlet at δ 2.9, upfield from the $N(CH_3)_2$ signals at δ 3.09.



Scheme 18: Synthesis of pentamethylmelamine and its deuterated analogue. Reagents: i, Me_2O, H_2O/ice then 20°C, $(CH_3)_2NH$; ii, H_2O, CH_3NH_2, NaOH, heat; iii, H_2O, $CD_3NH_3^+Cl^-$, NaOH, heat.

A novel deuterated **91** derivative was also synthesised for the structural investigation (assignment of the ^{1}H NMR spectrum) of the conjugate formed between pentamethylmelamine **90** and the indoledione **11**. The CD_3 compound **91** was prepared using trideuteromethylamine. In the ^{13}C NMR spectrum, the $^{13}CD_3$ signal was seen as a septet with a one-bond coupling constant $J_{C\text{-}D}$ = 20.8 Hz. The mass spectra were consistent with the NMR data. This deuterated isotopomer may also have applications in drug metabolism studies, in addition to its planned use here.

6.3.3 *Coupling of indolediones with isoquinolin-1-ones and with pentamethylmelamine.*

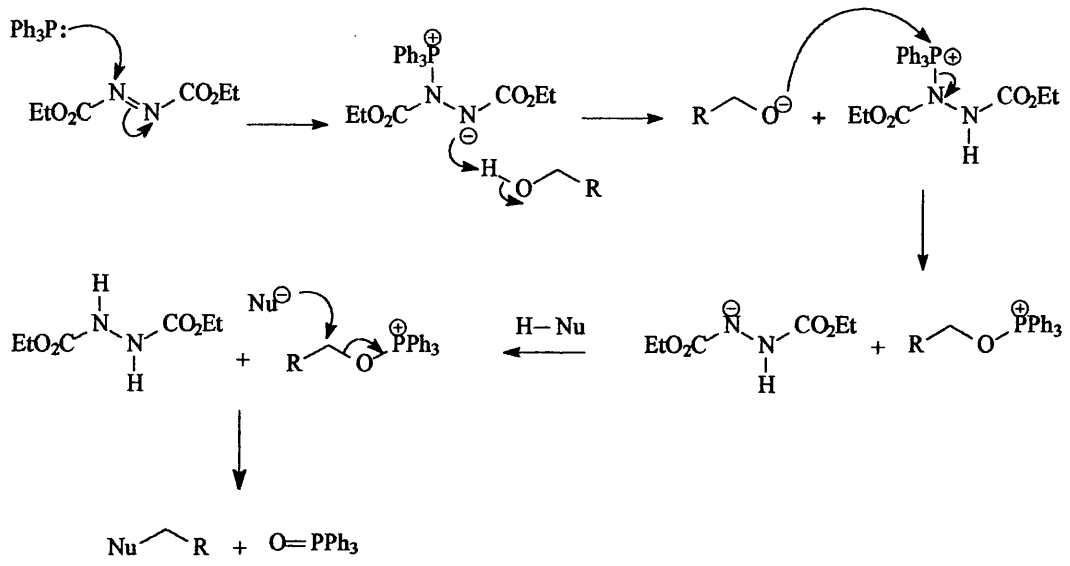
Alkylation of the anions derived from isoquinolin-1-ones usually takes place at the 2-nitrogen.[80,83,121,122] For example, Berry *et al*[83] and Parveen *et al*[80] deprotonated 5-bromoisoquinolinone with lithium bis(trimethylsilyl)amide and treated the nucleophilic species with chloromethylheterocycles. Alkylation occurred at nitrogen showing that this base deprotonated effectively the isoquinolinone used. Pentamethylmelamine **90** has also been converted into its anion which can be alkylated at the exocyclic secondary amine.[123]

Reaction between 3-(chloromethyl)indoledione **63** and isoquinolin-1-one **28** or pentamethylmelamine **90** under these conditions did not give the conjugates expected but only led to recovery of the starting materials. Investigation of the leaving group, solvent and base effect on the reactivity of the trigger, as previously discussed (part **6.2**), did not affect the course of the reaction.

However, Naylor *et al*[51] used Mitsunobu-type reactions for building up different groups at position 3 of the indole nucleus from **11**. The Mitsunobu reaction is known to allow the replacement of alcohols by nitrogen functions. Pentamethylmelamine possesses an exocyclic secondary amine. Isoquinolin-1-one contains a secondary amide motif which is essential for its pharmacological activity.[92] Therefore, to produce potential prodrugs, these secondary amine/amide motifs would have to be masked. Such masking could be achieved by coupling to the trigger moiety (carrying a primary alcohol) through the Mitsunobu procedure.

6.3.3 1 Mitsunobu reactions

The Mitsunobu reaction is a very versatile and useful method in organic synthesis which enables the replacement of an hydroxyl group by a wide range of nucleophiles.[124]



Scheme 19: Mechanism of the Mitsunobu reaction.

Addition of the phosphine to the weak N=N π bond is the first step of this reaction that gives the quaternary phosphonium salt with a nitrogen anion stabilised by one of the ester groups. This anion is sufficiently basic to remove the proton from the alcohol. The affinity between oxygen and phosphorus promotes the attack of the newly formed alkoxide ion on the positively charged phosphorus displacing the second nitrogen. The result of this reaction is the formation of an alkoxyphosphonium salt and of the basic nitrogen anion. The nitrogen anion can deprotonate the nucleophile, which can, in turn, attack the alkoxyphosphonium derivative *via* an S_N2 reaction with phosphine oxide as a leaving group (**Scheme 19**). The S_N2 mechanism also implies inversion of configuration at the electrophile. This diethyl azodicarboxylate / triphenylphosphine reaction system falls under the general category of redox reactions, since triphenylphosphine is oxidised to triphenylphosphine oxide and diethyl azodicarboxylate is reduced to diethyl hydrazinedicarboxylate.

Amongst many other possibilities, the transformation of an alcohol into an amine is one of the interesting options offered by the Mitsunobu reaction. Primary and secondary alcohols can be converted to amines and amine derivatives. The pKa of the acid (nucleophilic) component has been shown to be a key in the success of those reactions. Typically it has to be less than 14. This explains partly why this reaction is essentially used for the formation of carbon-heteroatom bonds. Mitsunobu reactions with carbon acid participants have been explored for the formation of carbon-carbon bonds. The choice of the phosphine / azo-derivative system can also influence the reaction. Henry *et al*[125] and Edwards *et al*[126] reported the synthesis of protected amines and secondary amines from alcohols *via* the Mitsunobu reaction.

Other alternatives are known to obtain this transformation, Murahashi *et al*[127] reported a ruthenium-catalysed system allowing the synthesis of secondary and tertiary amines from alcohols. Grigg *et al*[128] reported the use of other transition metals for the catalysis of such reactions.

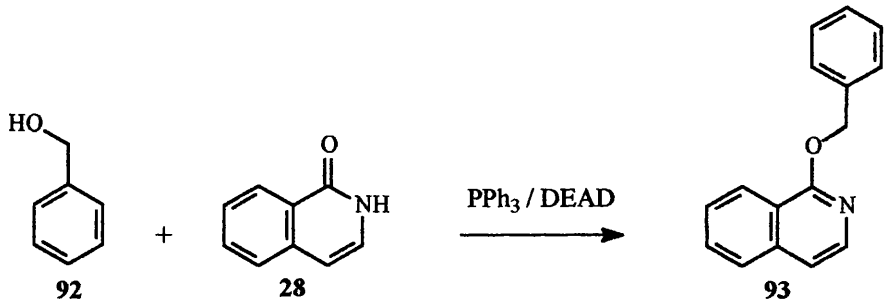
Diethoxytriphenylphosphorane (DTPP) has also been used in the more specific example of aziridine formation from 1,2-aminoalcohols[129].

Indole chemistry has been influenced, as well, by this type of reaction from the building up of substituents to the formation of the indole ring. Castro *et al*[130] reported the alkylation of sulfonamides with an hydroxymethyl group at position 5 of the indole ring. Bhagwat and Gude[131], Kaneko *et al*[132] and Ohkubo *et al*[133] used the indole as the nucleophilic moiety. Bhagwat and Gude[125]obtained a range of low to reasonable yields depending on the substituents on the indole ring. Activation of the NH was optimum when two electron-withdrawing groups were found at positions 2,3 or 2,5. Ohkubo *et al* [133] studied the nature of the phosphine / azo derivative system as a means to improve the yield of the indole N-alkylation. Macor and Wehner[134] used the reaction for building up the 5-membered ring of the indole; 2-(5-methoxy-2-nitro-phenyl)acetonitrile was deprotonated with a DEAD / PPh_3 complex and treated with the desired alcohol. The product obtained was hydrogenated and subjected to heat and gave the corresponding indole.

6.3.3.2 Model reactions following the Mitsunobu principle

As noted above, Berry *et al*[83] and Parveen *et al*[80] tested their alkylation methodology on a model system which consisted of deprotonating isoquinolin-1-one and selected 5-substituted isoquinolin-1-ones and alkylating the anions with (bromomethyl)benzene and its *para* substituted analogues. Alkylation took place exclusively at nitrogen in each case.

In the present work, it was attempted to reproduce this simple model using the Mitsunobu reaction to give information on the nucleophilic reactivity of the isoquinolin-1-ones. The first model Mitsunobu reaction was carried out using benzyl alcohol **92** and isoquinolin-1-one **28** (**Scheme 20**) DEAD and PPh_3. A colourless oil was obtained, as reported[83] for 2-benzylisoquinolin-1-one, but proton NMR showed different chemical shifts compared to the ones reported for this N-alkylated material.[83]

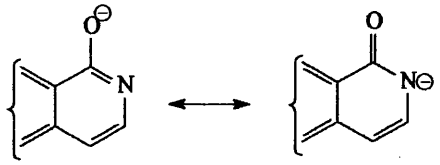


92 + **28** → **93**

Scheme 20: Model reaction between benzyl alcohol and isoquinolin-1-one. Reagents: i, PPh_3, DEAD, THF.

In particular, the signal for the CH_2 was observed further downfield than expected: shifting from δ 5.20 to δ 5.58. The H-3 resonance was noticeably shifted downfield, from δ 7.05 to δ 8.00. The H-4 and H-5 signals followed the same trend, although not as profoundly as that of H-3. The mass spectrum gave the expected mass ion, with a similar fragmentation pattern to that of the N-benzyl compound. The deshielding effects observed together with the MS evidence led to consideration of a different structure for the compound. The benzyl moiety was alkylated on the oxygen of the isoquinolinone and not on the nitrogen, giving 1-benzyloxyisoquinoline **93**. This compound has acquired fuller aromaticity in the heterocyclic ring, causing the major

deshielding of H-3 and H-4 and the milder effect on the protons of the fused benzene ring. Further evidence for O-alkylation was provided by the ^{13}C NMR spectrum, in which the methylene carbon signal was at δ 69.4, showing that it is of the OCH_2 type, rather than in the region δ 30 - δ 40 typical of $PhCH_2N$ carbons. Mitsunobu reactions on isoquinolon-1-ones are previously unknown but Manhas *et al*[135] reported that treatment of 2-arylquinazolin-4(*3H*)-ones with propan-2-ol and with 2-(pyrrolidin-1-yl)ethanol in the presence of DEAD and PPh_3 gave the corresponding ethers in high yields. Similarly, pyrimidine-2,4-dione, when treated with cholestan-3α-ol gave the corresponding diether. The method did not lead to ether formation when tertiary alcohols were used. Studies on conjugated amides also proved unsuccessful in forming the ether. Manhas *et al*[135] suggested that the reaction is due to the participation of the enolic form of the amide. No parallel formation of the ether and N-alkyl derivative in any one reaction was reported. Investigations on solvent effect and order of addition of the reagents proved to have no influence on the course of the reaction. However, this suggestion must be regarded sceptically, since the actual reactive nucleophilic intermediate is the corresponding anion, which is not tautomeric **(Scheme 21)**.

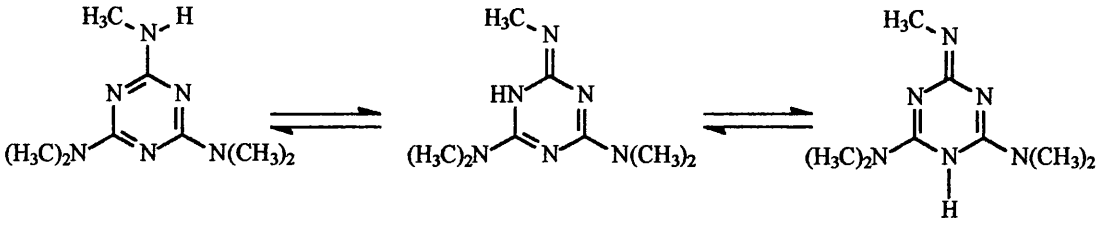


Scheme 21: Resonance forms of the isoquinolinone anion generated in the Mitsunobu reaction.

The evidence that isoquinolinone could be used as a nucleophile in the Mitsunobu reaction was given with the formation of **93**. The next step was to demonstrate that the indoledione **11** could be used as a potentially electrophilic alcohol.

The model reaction for that was based on the use of pentamethylmelamine (PMM) as the nucleophilic component. The amine targeted as the attachment site was relatively free of steric hindrance and is likely to have a pKa less than 14. The reaction was carried out under the conditions used for the formation of **93**.

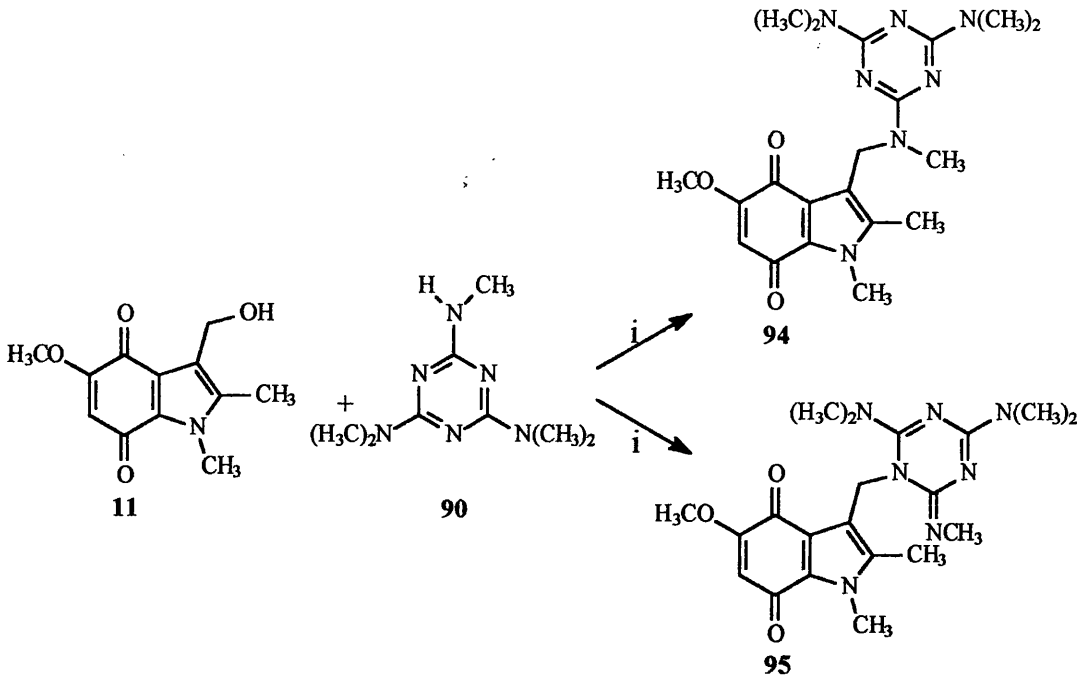
Proton NMR analysis of the product formed showed interesting results. The signal assigned as the CH_2 group was observed further downfield than expected: δ 6.42 indicating that these methylene protons (on an sp^3 carbon) were in a more deshielding environment than the aromatic indole proton H-6 (on an sp^2 carbon). The signal was also a multiplet, suggesting a conformation rigid enough for the molecule to be unable to rotate and rendering the two protons magnetically inequivalent. The possible attachment sites of **11** on the **90** were considered.



Scheme 22: Tautomeric forms of pentamethylmelamine.

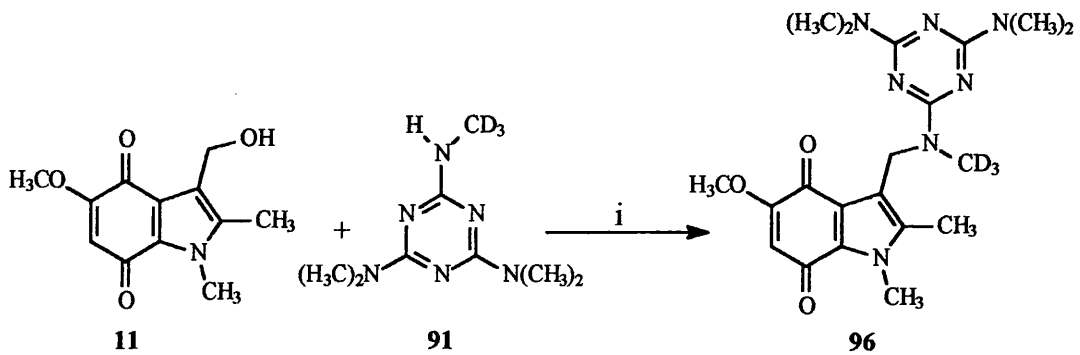
It is known that PMM exists in other tautomeric forms in which it is relatively stable, although crystal structures of PMM from the literature report the structure with exocyclic $NHCH_3$[119] (**Scheme 22**). In the intermediate anion, the negative charge may be delocalised onto the ring nitrogen atoms. In other words, in the alkylated product, the indole could be bound to one of the ring nitrogens, explaining why the methylene would have such a chemical shift; the nitrogen being part of the aromatic ring would exert a greater electron-withdrawing effect than the exocyclic $NHCH_3$ on the CH_2 group (**Scheme 23**). The compound decomposed rapidly and no further analyses could be carried out.

Repeats of the experiment carried out subsequently did not give such chemical shifts, although the coupling reaction was taking place. This might suggest that the first reaction occurred to give a less stable product which then underwent rearrangement and migration of the substituent from the ring to the exocyclic nitrogen atom to give a more stable isomeric form. This was also considered to explain the non-repeatability of the first reaction.



Scheme 23: Synthesis of 1,2-dimethyl 3-{N-[[4,6-bis(dimethylamino)-1,3,5-triazin-2-yl]-N-methyl-amino]methyl}-5-methoxyindole-4,7-dione. Reagents: i, PPh_3, DEAD, THF.

Mitsunobu reaction between **11** and the deuterated PMM **91** gave the expected isotopomer **96** (**Scheme 24**). A moderate yield (40%) was obtained for both reactions. Temperature and order of addition were shown to have no influence.



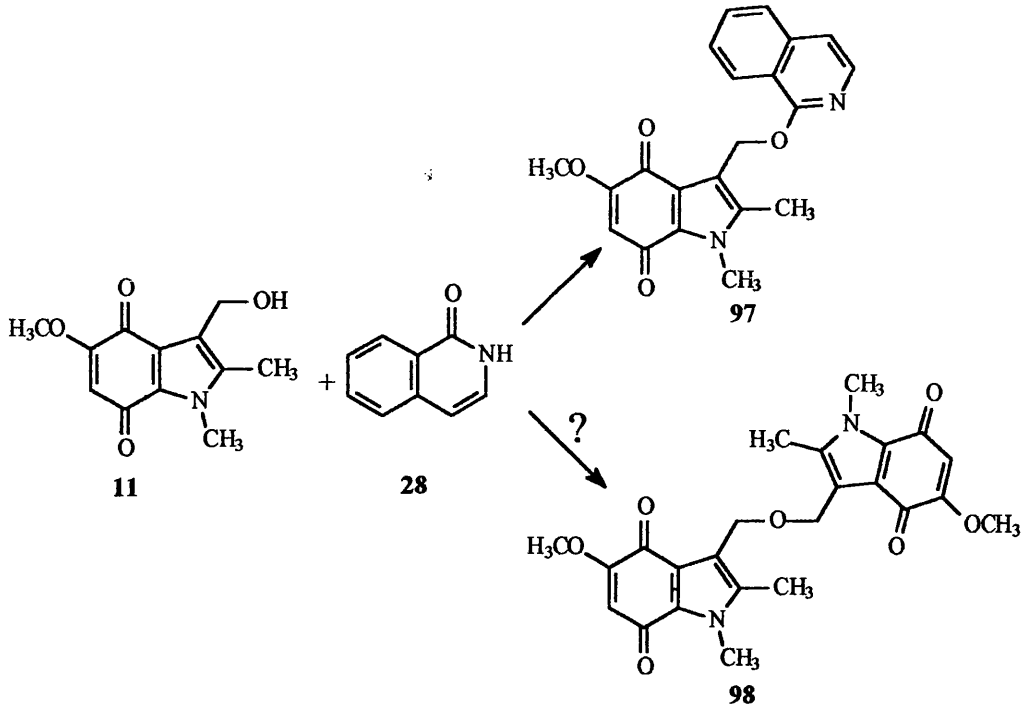
Scheme 24: Synthesis of **96**, the deuterated derivative of **94**. Reagents: i, PPh_3, DEAD, THF.

The deuterated derivative was designed to solve the problem of assignment of the NCH_3 signals in the ^1H NMR spectra. Typically, the ^1H NMR spectrum obtained for conjugate **94** showed the indole proton H-6 at δ 5.61. The CH_2 signal came at δ 5.12 which was more upfield than for the first compound obtained but the signal was still a multiplet, indicating the non-equivalence of the two methylene protons. CH_3O and CH_3N were observed as singlets at δ 3.85 and δ 3.81, respectively, followed by the melamine $N(CH_3)_2$ at δ 3.11 and CH_3N at δ 2.94, both as singlets. Similar chemical shifts were obtained for the deuterated derivative; the singlet at 2.94 ppm was missing since CD_3 gave no signal. NMR analysis of this derivative proved that the melamine CH_3N is more shielded then the indole N-methyl observed further downfield. Further evidence of the formation of the conjugates was obtained by mass spectrometric analysis.

Evidence for the reactivity of both trigger and drug under Mitsunobu reaction conditions were brought by the model reactions. The series of target compounds could then be synthesised by this method. Isoquinolin-1-one **28** was the first drug used in this series of reactions.

6.3.3.3 Mitsunobu reactions of indoledione **11** and isoquinolin-1-ones.

Treatment of **28** with the indoledione **11** in the presence of DEAD and PPh_3 gave two compounds, together with some starting material. Proton NMR identified the first compound as being the conjugate between **11** and **28**. The structure of the second compound could not be resolved but one may speculate that it could be the dimer **98** of alcohol **11** (**Scheme 25**).



Scheme 25: Mitsunobu reaction between isoquinolin-1-one and 1,2-dimethyl-3-(hydroxymethyl)-5-methoxyindole-4,7-dione. Reagents: i, PPh_3, DEAD, THF.

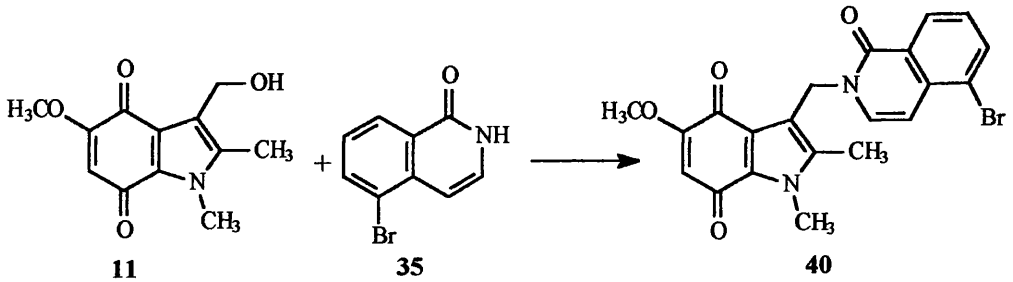
There was considerable difficulty in separating the products from the side-product diethyl hydrazinedicarboxylate. The literature[124] on the Mitsunobu reaction reports contamination and difficult purification due to the presence of triphenylphosphine oxide but diethyl hydrazinedicarboxylate is not mentioned. Colleagues noted (unpublished results) the formation of complexes between conjugates formed by the Mitsunobu reaction and diethyl hydrazinedicarboxylate. In the present study, recrystallisation from methanol enabled the isolation of the target compound.

The ^1H NMR spectra showed some interesting results, which correspond to the ones observed from **93**, obtained from isoquinolinone and benzyl alcohol. In the case of **97**, the methoxy, N-methyl and 2-methyl group from the indole moiety showed little changes in their chemical shift compared to the starting material. H-6 was still around δ 5.62, but the CH_2 group was observed further downfield, at δ 5.72, from δ 4.65 in the starting material, already showing important downfield shifting and suggesting linkage to oxygen. The isoquinoline protons were, as in the case of **93**, importantly

shifted downfield from their positions in the spectrum of isoquinolin-1-one. The H-4 signal resonated as a doublet at δ 7.2 followed by H-7 observed as a *pseudo* triplet, coupled to H-8 and H-6, at δ 7.45. H-6 was observed as a *pseudo* triplet at δ 7.61, H-5 as a doublet at δ 7.70 and was coupled to H-6. H-3 was 1 ppm downfield compared to its chemical shift in the starting material and was seen at δ 8.0. Finally, H-8 resonated as a doublet of doublets at δ 8.17, with a small *meta* coupling with 6-H. Similarly to **93**, the change in chemical shift affected H-4, H-3 and H-5 more than the three other protons. The ^{13}C NMR spectrum indicated the presence of CH_2 at δ 53.4, which suggests that this group is linked to the oxygen of the isoquinolinone. If it had been adjacent to the nitrogen atom, its chemical shift would have probably been seen about 20 ppm upfield. Mass spectrometric analyses confirmed the structure of the compound.

Isoquinolin-1-one was shown to have reduced activity against PARP compared to its 5-substituted analogues[80,83,92-94,109-110]. Three 5-substituted isoquinolin-1-ones were selected and synthesised to be linked to the indoledione trigger **11**, namely, 5-iodo-, 5-bromo- and 5-aminoisoquinolin-1-ones.

Synthesis of the bromo analogue **40** was carried out using the same reaction conditions as for **97** (**Scheme 26**).

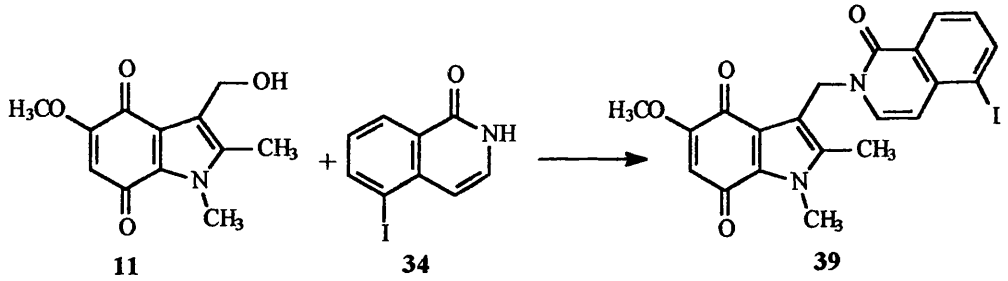


Scheme 26: Synthesis of 1,2-dimethyl-3-(5-bromo-1-oxoisoquinolinyl-2-methyl)-5-methoxyindole-4,7-dione. Reagents: i, PPh_3, DEAD, THF.

TLC analysis showed evidence for the formation of two compounds: one was the target compound **40**, the second one is speculated to be a dimer of the parent trigger. It was expected to obtain a similar ^1H NMR spectrum to **97**, without the signal from H-5

since that position of the indole ring was now substituted with a group having a negative inductive effect. However the CH_2 protons, 3- and 4-H from the isoquinoline were less deshielded than expected. The methylene protons resonated downfield from the indole 6-H, at δ 5.30. 4-H resonated slightly more upfield than in the previous conjugate, at δ 6.77. The *pseudo* triplet for 7-H was at δ 7.27, 6-H resonated as a doublet since it was only coupled to 7-H, at δ 7.82, so did 3-H. Finally, the signal for 8-H was observed at δ 8.36. ^{13}C NMR analysis showed the CH_2 group signal at δ 32.6, indicating that the trigger was linked to the nitrogen of **35** instead of the oxygen as previously seen for compounds **93** and **97**.

The following analogue synthesised was the 5-iodo analogue of **97**, compound **39** (**Scheme 27**).



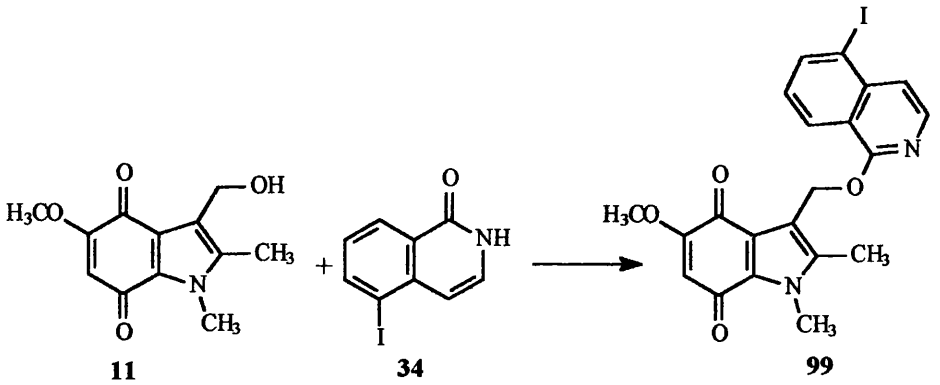
11 **34** **39**

Scheme 27: Synthesis of 1,2-dimethyl-3-(5-iodo-1-oxoisoquinolinylmethyl)-5-methoxyindole-4,7-dione. Reagents: i, PPh_3, DEAD, THF.

^1H NMR analysis showed that the chemical shifts observed for **39** corresponded to the ones obtained for **40**. The 6-H and 3-H signals were observed, respectively, at δ 8.01 and δ 7.8, suggesting that the dioxoindole-CH_2 is also linked at the nitrogen atom in **39**.

However, a repeat experiment under apparently the same conditions led to compound **99** which had the same Rf as **39** on TLC analysis but had a different appearance and exhibited different NMR results (**Scheme 28**). In the ^1H NMR spectrum, 8-H was observed at δ 8.12, followed by 6-H and 3-H at δ 8.15 and δ 8.08, respectively. 4-H was observed at δ 7.40, downfield from 7-H at δ 7.16. The methylene protons were observed at δ 5.71 instead of δ 5.62, downfield from 6'-H. The chemical shift of the

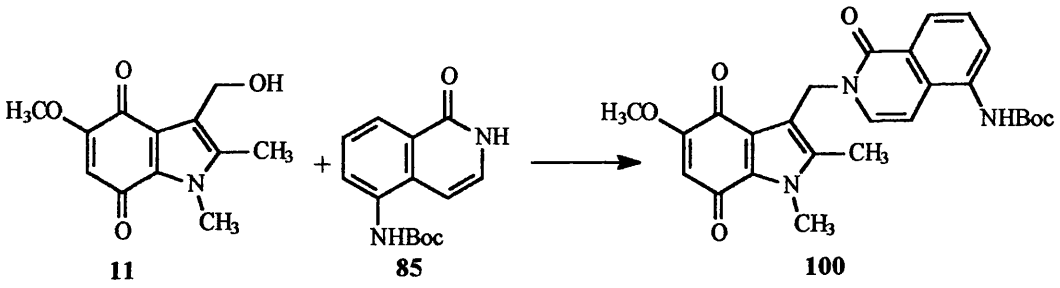
methyl group suggested that the compound was linked, more probably, to the oxygen than to the nitrogen of the isoquinolinone due to the deshielded character of CH_2 and 3- and 4-H. Mass spectroscopic analysis showed the same mass ion for **99** and **39**. It is interesting to note that 3-H and 4-H were the two protons that seemed the most affected, suggesting O-alkylation. ^{13}C NMR showed an important shift downfield of the CH_2 group of approximately 20 ppm compared to the chemical shift of the CH_2 group for **39**, explained by the difference in electronegativity between nitrogen and oxygen and confirming the hypothesis.



11 **34** **99**

Scheme 28: Synthesis of 1,2-dimethyl-3-(5-isoquinolin-1-yloxymethyl)-5-methoxy-indole-4,7-dione. Reagents: i, PPh_3, DEAD, THF.

Finally, conjugate **100** was prepared from the Boc protected aminoisoquinolinone **85**. The reaction conditions were similar to the ones previously used (**Scheme 29**) for **39**, **40**, **97** and **99**.



11 **85** **100**

Scheme 29: Synthesis of compound **100**.

Evidence for the formation of this compound was observed by TLC analysis and proton NMR. The chemical shifts obtained matched the ones of compounds **40** and **39**, indicating that the indoledione is alkylated onto the nitrogen atom of the isoquinolinone derivative in **100**. The low yield of the reaction did not allow the subsequent deprotection of the amine. The conjugate **100** decomposed on mass spectrometric analysis.

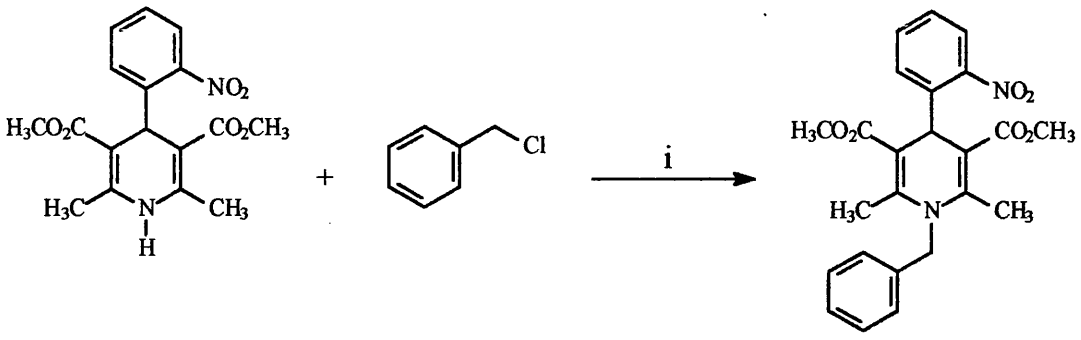
In this part, the strategy and synthesis of a series of analogues based on an indoledione trigger **11** and some 5-substituted isoquinolinone PARP inhibitors were discussed. The method used was selected after it was shown that simpler and more classical methods were not appropriate. The yields are uniform throughout the four reactions; however, the alkylation pattern observed remains unexplained. Temperature, concentration and order of addition were shown to have no influence on the course of the reaction. Further studies could be carried out on the improvement of the yield using this method and possibly modifying the nature of the Mitsunobu complex[136].

6.4 *APPROACHES TO POTENTIAL PRODRUGS WITH 1,2-DIMETHYL-5-METHOXYINDOLE-4,7-DIONE TRIGGER AND NIFEDIPINE EFFECTOR.*

Nifedipine **101** is known to be a calcium channel blocker from the family of the dihydropyridines. It was initially selected to be one of the drug moieties for prodrug-mediated delivery. Co-delivery of nifedipine with the active substance would potentially ensure that the drug delivered would remain trapped in the site of delivery.

The first approach to link indoledione to nifedipine **101** was to deprotonate the NH group of the drug to form a nucleophile that would displace the chloride leaving group attached to the CH_2 group in **63**, as investigated for prednisolone and isoquinolin-1-one. The reaction was carried out in the presence of sodium hydride as a base. No reaction was observed. The choice of the base was critical; sodium hydride readily decomposes in the presence of any trace of moisture and it is difficult to handle. It is also known to promote the decomposition of the starting materials in some cases.

An alternative was to use lithium bis(trimethylsilyl)amide as a base. However, the initial small supply of the indoledione **63** motivated the use of a model reaction with benzyl chloride. The reaction between nifedipine **101** and benzyl chloride was successful with a yield of 40%. (**Scheme 30**)

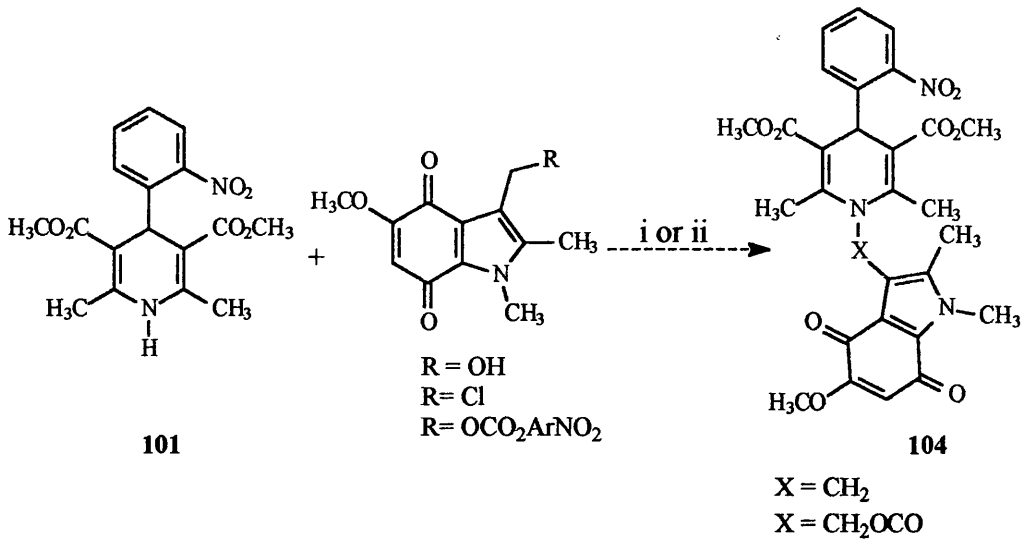


101 **102** **103**

Scheme 30: Model reaction between benzyl chloride and nifedipine. Reagents: i, $LiN(SiMe_3)_2$, NaI, DMF.

Repeating the reaction with **63** instead of **102** did not lead to the expected conjugate. Explanations for this lack of reactivity are not obvious. Steric hindrance was the main factor considered.

As for the prednisolone series, attempts were then made to link the indoledione to the nifedipine *via* a carbonyl group, in this case a carbamate. Remy *et al*[137] reported acylation of a series of dihydropyridines using sodium hydride in THF and methyl chloroformate, forming the methyl carbamate. However, treatment of nifedipine **101** with the nitrophenyl carbonate **64** gave no apparent reaction (**Scheme 31**).

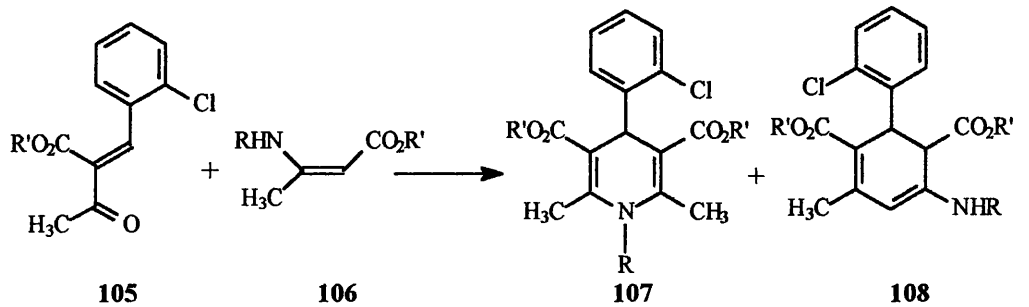


Scheme 31: Attempted syntheses of conjugate **104** from **101** and derivatives of **11**.

Reagents: i, NaH or $LiN(SiMe_3)_2$, NaI, THF, Ar; ii, for X_1 only, DEAD, PPh_3, THF.

Finally, the Mitsunobu reaction was attempted but variations in concentration of the Mitsunobu complex, temperature or order of addition did not lead to N-alkylation of the nifedipine.

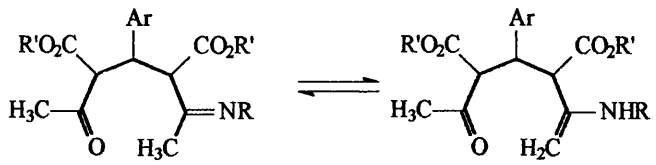
Another way of approaching the problem would have been to "build" the dihydropyridine using the Hantzsch synthesis of 1,4-dihydropyridines **(Scheme 32).** The bicyclic molecule can be prepared from the condensation of N-alkyl β-amino-crotonates and benzylidine acetoacetic esters. Patterson[138] showed that the precursor aminocrotonate can be N-alkylated before condensation using groups as bulky as benzyl groups. This example suggests that N-alkylation by the indole group could lead to the target molecule using this method.



105 **106** **107** **108**

Scheme 32: Patterson's model of the Hantzsch synthesis of N-alkylated 1,4 dihydropyridines[138].

Formation of side products, such as the cyclohexadienamine **108**, formed through cyclisation of the intermediate enamine with the carbon rather than the nitrogen (**Scheme 33**), has been reported by Patterson[138] together with low yields.

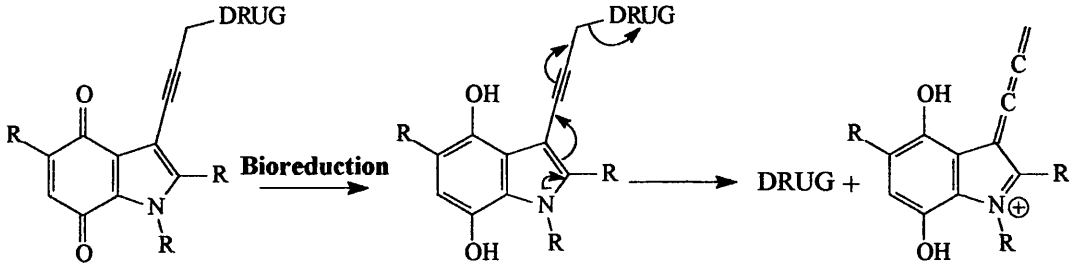


Scheme 33: Tautomeric forms of the intermediate in the Hantzsch synthesis of N-alkylated 1,4-dihydropyridines.

Change in solvents often solved the reported problems. Ethanol favoured the formation of the side product whereas non-protic solvents, such as benzene or toluene, reversed the ratio of products[138].

7. DESIGN OF A NEW INDOLE TRIGGER

The idea motivating the synthesis of a new trigger was to allow the release of the drug as efficiently as possible after reductive activation of the trigger. The nature of the linker was thought to have a noticeable effect on the activation potential and release efficiency. Modification and synthesis of a new trigger permitting synthesis of a series of potential prodrugs similar to the one discussed in part **6.0** would have been a valuable element of comparison. The extension of the conjugated system by the addition of the triple bond at position 3 of the indole ring was the first alteration planned.



Scheme 34: Proposed mechanism for the bioreductively triggered release of drugs from indoledione-3-propynyl potential prodrugs.

The formation of a four-carbon cumulene after potential reduction and release of the drug is a valid hypothesis since the formation, isolation and characterisation of such compounds has already been reported.[139]

The main difference in the synthetic route initially designed compared to the route to **11** was the Heck-Castro coupling of an acetylene group at position 3 of the indole nucleus. The Heck-Castro coupling reaction is a palladium-copper catalysed reaction of vinyl or aryl halides with terminal acetylenes. The reaction proceeds *via* the copper alkynide formed *in situ* as represented below (**Figure 21**). Usually the coordinatively unsaturated catalysts $Pd(PPh_3)_2Cl_2$ and $Pd(MeCN)_2Cl_2$ are more active than $Pd(PPh_3)_4$. Among the bases, piperidine is particularly effective but triethylamine and diethylamine are also common. As a copper source, CuI or CuBr is almost always used.

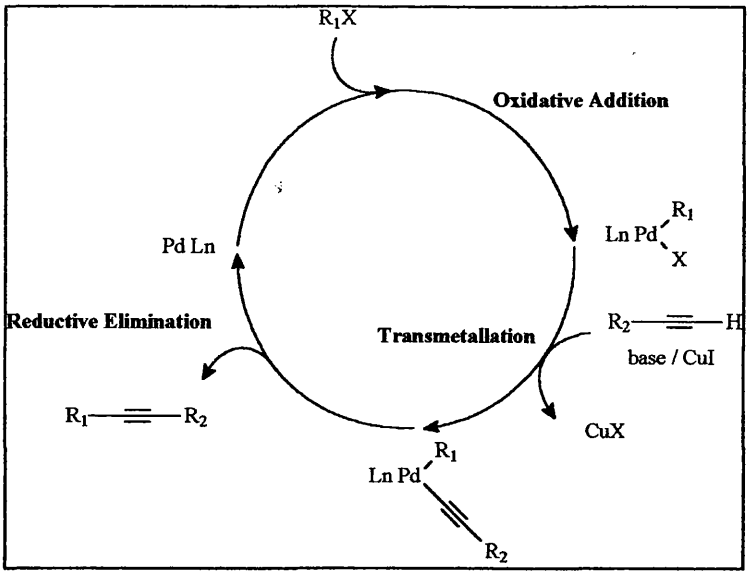


Figure 21: Heck-Castro coupling mechanism.

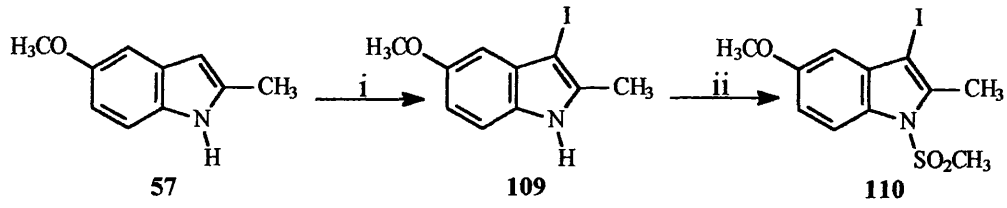
To achieve the coupling, it was necessary to iodinate the indole at position 3. 1,2-Dimethyl-5-methoxyindole **58** was initially chosen as the starting material. Reaction of **58** with iodide monochloride in the presence of pyridine did not give the expected iodinated derivative. The literature[104] suggested that the nature of the N-substitution at position 1 would explain this phenomenon. In the absence of substituent (R = H) or if an electron-withdrawing group is present (reducing electron density, thus limiting poly-iodination), iodination at position 3 should occur with reasonable yield.

The same reaction was carried out with 2-methyl-5-methoxyindole **57**, affording the iodinated compound but with a relatively low yield. The instability to air and light of **109** made it difficult to purify.

The iodinated derivative **109** was used for the Heck-Castro coupling reaction using two different acetylenes, namely propargyl bromide and propargyl alcohol, and two different palladium catalysts (tetrakis(triphenylphosphine)palladium and dichlorobis-(triphenylphosphine)palladium) and copper iodide under basic conditions. No reaction was observed, the starting material was recovered.

Sakamoto *et al*[140] reported a series of palladium-catalysed coupling reactions of 3-iodoindoles, possessing an electron-withdrawing group at position 1 or 2, with terminal acetylenes. Starting from an unsubstituted indole ring, in the presence of potassium hydroxide, and iodine, selective iodination took place at position 3 of the

indole ring, which is the most reactive to electrophilic attack. 3-Iodoindole was poorly stable during purification and was therefore treated with methanesulfonyl chloride under basic conditions to give 1-methylsulfonyl-3-iodoindole, which was more stable and could therefore be purified and undergo the subsequent palladium-catalysed reaction.



57 109 110

Scheme 35: Synthetic pathway to 3-iodo-5-methoxy-2-methyl-1-(methylsulfonyl)-indole. Reagents: i, I_2, KOH, DMF; ii, $MeSO_2Cl$, NaOH, Bu_4NBr, benzene, water.

A similar protocol was followed for the preparation of **110** (**Scheme 35**). The relatively low yield obtained for both steps is suggested to be due to the presence of the electron-donating methyl group at position 2. The electron-withdrawing group on the nitrogen atom is responsible for the improved stability of the molecule, as reported by Sakamoto *et al.*[140]

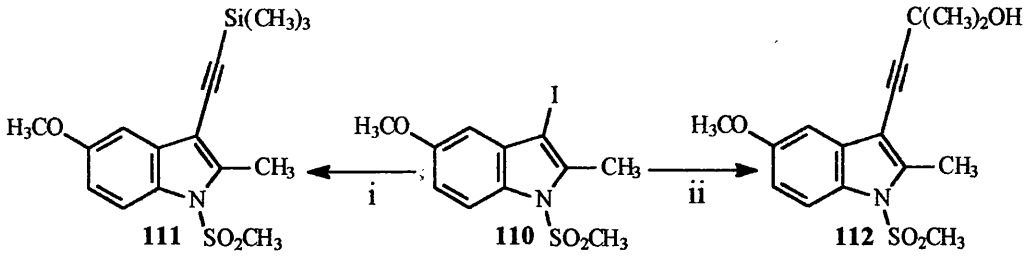
Proton NMR analysis of the first two compounds showed some changes in chemical shift of the aromatic protons as electron-withdrawing substituents were inserted. After iodination at position 3 of the indole ring, the methyl and methoxy protons previously seen as two singlets at δ 2.37 and δ 3.80, respectively, remained unchanged. H-3 observed at δ 6.10 in the starting material was not present in the iodo-derivative. H-6 came as a doublet of doublet due to *ortho* coupling with H-7 and *meta* coupling with H-4, at δ 6.73 in both the starting material and 3-iodo-5-methoxy-2-methyl-1 *H*-indole. H-7 seen as a doublet at δ 7.18 in the precursor indole remained unchanged after iodination. H-4, observed as a singlet, shifted from δ 7.00 to δ 7.99 after iodination due to the negative inductive effect of iodine. The broad singlet seen for NH also shifted from δ 7.71 to δ 8.52.

After N-methylsulfonylation, the singlet for SO_2CH_3 appeared at δ 3.00 between the methyl and methoxy group at δ 2.68 and δ 3.80. H-4 is shifted back upfield at δ 6.84. The methylsulfonyl group is known to be electron-withdrawing but its position triggered the deactivation of the whole pyrrole ring, presumably overcoming any magnetic effect from the iodine on the fused benzene ring. The H-6 signal came at δ 6.95 and H-7 was observed downfield compared to its previous position, at δ 7.85, illustrating the deshielding effect of the N-methylsulfonyl group.

The following step involved the coupling of the iodine derivative with selected acetylene derivatives. The choice of the latter was based on the observations made during the different attempts to attach the indoledione trigger **11** to the selected drugs. Propargyl bromide was thought to be a good choice for future nucleophilic substitutions together with propargyl alcohol for its versatility. The former would lead to an indoledione unit with a reactive electrophile, whereas the indoledione-propargyl alcohol would be a useful component of a Mitsunobu coupling. Coupling experiments proved that propargyl bromide was unreactive under the coupling conditions used. Sakamoto *et al*[140] reported the inertness of propargyl alcohol in this kind of coupling experiments with indoles.

Alternative acetylenes were selected, introducing the desired versatile functional groups: 2-methyl-3-butyn-2-ol and trimethylsilylacetylene[140]. 2-Methyl-3-butyn-2-ol would potentially afford an alcohol derivative, as it was desired, but the introduction of steric hindrance with the two methyl groups would also be a potential toxicity-limiting factor to observe in the case of *in vivo* studies. The TMS derivative would be easily transformed into other types of leaving groups and was therefore a convenient starting point.

Coupling reaction were carried out under strong basic conditions in the presence of a mixture of the acetylene, **110**, CuI and $Pd(PPh_3)_2Cl_2$. The reaction with trimethylsilyl-acetylene afforded **111** in 60% yield (**Scheme 36**).



Scheme 36: Heck-Castro coupling of 3-iodo-5-methoxy-2-methyl-1-methylsulfonyl-indole with 2-methyl-3-butyn-2-ol and trimethylsilylacetylene. *Reagents*: i, CuI, $Pd(PPh_3)_2Cl_2$, DMF, Et_3N, trimethylsilylacetylene, Ar; ii, CuI, $Pd(PPh_3)_2Cl_2$, DMF, Et_3N, 2-methyl-3-butyn-2-ol, Ar.

^{1}H NMR analysis brought evidence for the formation of the compound. For compound **111**, H-7 was seen as a doublet at δ 7.83, followed by H-4, a narrow multiplet, at δ 7.05, and H-6 (δ 6.94) as a doublet of doublets, for it is coupled to H-4 and H-7 (coupling constants are typical of *ortho* and *meta* coupling, J = 9.1 and 2.5 Hz). The methoxy, methylsulfonyl and 2-methyl groups were observed as singlets at δ 3.88, δ 3.01, and δ 2.69, respectively. The singlet expected for the nine protons of the trimethylsilyl group was observed at δ 0.3 due to the deshielding effect of the triple bond.

Compound **112**, obtained in 60% yield, showed similar splitting patterns and chemical shifts in the NMR spectrum. The six protons of the geminal dimethyl unit were observed at δ 1.69 as a single peak.

The following steps planned for both compounds were the deprotection of the nitrogen atom, N-methylation and building of the paraquinone on the 6-membered ring by nitration, reduction to the amine, and oxidation to the paraquinone.

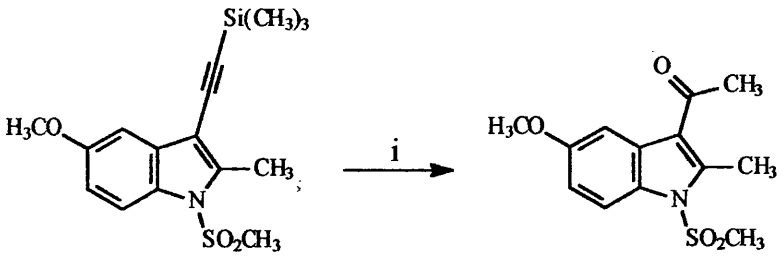
Deprotection of 2,3-substituted-N-alkylsulfonylindoles was reported by Yasuhara and Sakamoto[141] and involved the use of one equivalent of tetrabutylammonium fluoride (TBAF) in THF under reflux from 30 to 90 min. The method is mild and fast, compared to the more commonly used basic hydrolysis. The yields reported ranged from 38% to 100%. Other methods are known, such as five equivalents of K_2CO_3 in

refluxing aqueous methanol[142] and 5 M NaOH in methanol,[143] or reductive conditions like Na-Hg in NaH_2PO_4-buffered EtOH.[143]

Sundberg and Laurino[142] used 5% methanolic potassium carbonate and 18 hours reflux to deprotect a series of indoles monosubstituted on the 6-membered ring with a variety of substituents. The reported yields were high, ranging between 85% and 98%. The methods used are thus generally harsh, and the yields are not always as good as the ones given as examples above. The nature of the substituent on the sulfonyl influences the ease of deprotection. Substituents on the pyrrole ring are certainly determining the degree of lability of those groups, especially at position 2.

The first attempt of deprotection of **111** by the Yasuhara and Sakamoto[141] failed and the starting material was recovered. Increased TBAF concentrations together with increased temperature and reaction time did not influence the cleavage of the methylsulfonyl group. The method involving five equivalents of potassium carbonate also proved to be unsuccessful. The reaction was repeated since Ravikanth *et al*[144] reported that such conditions would have cleaved the TMS group at the same time to afford a terminal alkene. No cleavage of the TMS group was observed.

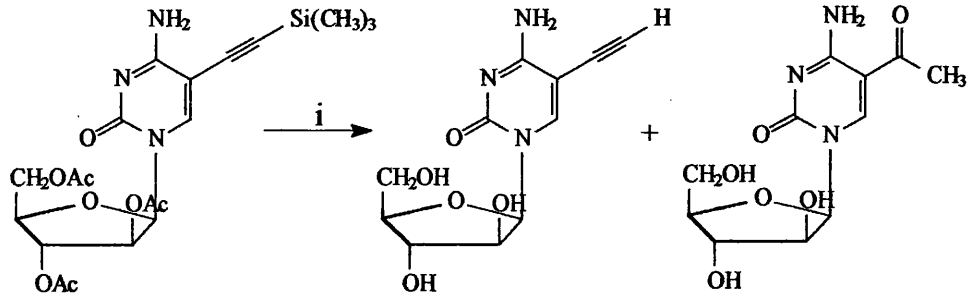
Treatment of **111** with methanolic potassium hydroxide gave a new compound detected by TLC analysis (**Scheme 37**). Proton NMR showed a broad peak integrating for 1 H at δ 8.40 recognised as the NH group. The indole H-4 was detected at δ 7.60, followed by H-7 at δ 7.21 and H-6 at δ 6.85. Three single peaks integrating for three protons were found at δ 3.88, δ 2.73, and δ 2.71, respectively. The first and the last peaks were assigned as the methoxy and the methyl group initially present on **111**. No sign of the nine $Si(CH_3)_3$ protons was observed. Mass spectrometry confirmed the reduction in mass of the compound and showed a fragment corresponding to a methyl carbonyl (acetyl) group. Calculation of the mass with this fragment integrating the groups identified on the proton NMR gave the mass found for the unknown compound on the mass spectrum. The chemical shift of the unidentified peak on the proton NMR was also corresponding to the one of a methyl ketone.



111 **113**

Scheme 37: Reaction of **111** under refluxing basic conditions. Reagents: KOH, MeOH.

Hydration of an aromatic alkyne to obtain a methyl ketone is a known reaction taking place under *acidic* conditions, catalysed by mercury(II) sulfate, or mercury(II) oxide.[145,146] Bobek *et al*[147] reported the deprotection of an heteroaromatic alkyne, namely 1-(2,3,5-tri-O-acetyl-β-D-arabinofuranosyl)-5-[2-(trimethylsilyl)ethynyl]-cytosine, from its trimethylsilyl protecting group by using anhydrous potassium carbonate in methanol at 20°C for 5 hours. The deprotection proved to be successful but 5-acetyl-1-β-D-arabinofuranosylcytosine was also detected (**Scheme 38**). Speculations on the eventual hydration of the ethynyl group were the only explanation for this reaction.

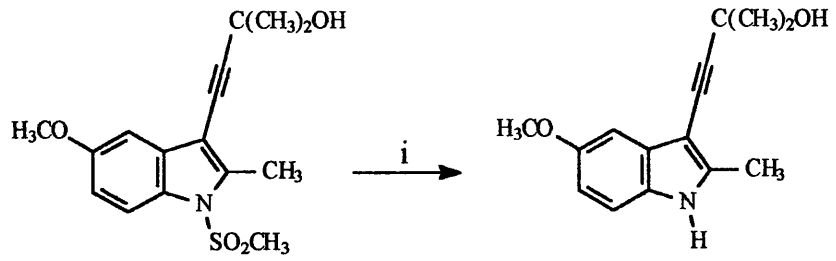


114 **115** **116**

Scheme 38: Basic deprotection of 1-(2,3,5-tri-O-acetyl-β-D-arabinofuranosyl)-5-[2-(trimethylsilyl)ethynyl]cytosine reported by Bobek *et al*[147]. *Reagents*: K_2CO_3, MeOH, 5 h.

The alcohol analogue **112** was subjected to the same deprotection methods as was **111**. The potassium carbonate, potassium hydroxide and TBAF methods were run in

parallel and the first two runs did not lead to deprotection. Repeating the reactions with increased temperature and reaction time led to the first deprotection *via* the TBAF method (**Scheme 39**). 5-Methoxy-2-methyl-3-(3-hydroxy-3-methyl-1-butynyl)-indole **117** was afforded in 50% yield. TLC analysis and proton NMR proved the presence of the compound, backed up by mass spectrometric analysis.



112 **116**

Scheme 39: Deprotection reaction of **112** under refluxing basic conditions. *Reagents*: TBAF, THF, Ar.

In the absence of the strong deshielding effect from the methylsulfonyl group, the chemical shift of H-7 was observed further upfield at δ 7.15, H-4 at δ 7.03 and H-6 at δ 6.79 were not affected. The broad single signal for NH came at δ 8.16. No change in the chemical shift of the aliphatic protons was noted. The methylsulfonyl singlet was not present in the spectrum.

The reaction proved to be non-repeatable; under similar conditions, the reactions would either work and give very variable yields and most of the time not work at all for reasons that are still unclear.

The last approach was to try and build the paraquinone before deprotection. Speculation were made on the deshielding effect of the *para* carbonyl group that could have, eventually rendered the sulfonyl protecting group more labile. Sulfonyl protecting groups are strong electron-withdrawing groups, as it has been mentioned above. The sulfonyl group at position 1 of the indole deactivates the pyrrole ring, making it fairly unreactive to electrophilic attack and oxidation[104]. It may affect the fused benzene ring[104]. Further investigation would be necessary to find a suitable

deprotecting system for the N-methylsulfonyl. The study of other electron-withdrawing protecting groups could also be a different way of solving the problem.

A series of indoledione based potential prodrugs have been synthesised. The synthesis of a new trigger containing an 3-alkene substituent was not achieved. However, some interesting aspects of indole chemistry have been highlighted. The diversity of the structure of those seven targets (difference in the linkage unit, drug substitution effect) was expected to be reflected in the results of the release studies.

8. NITROTHIOPHENE TRIGGER

Nitrothiophene was selected as a trigger for its ease of reduction, its structural similarity with nitroimidazole and because it was shown to be very efficiently activated under reductive conditions *in vitro* in the study reported by Threadgill *et al.*[86]

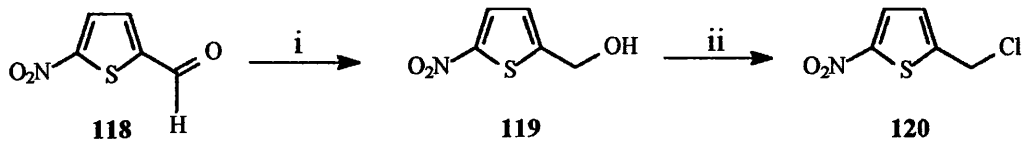
Literature about nitroimidazoles[80,82] and nitrofurans[83,84] used as triggers suggested two main ways of approaching the synthesis of the potential prodrugs. The first one was *via* the use of (5-nitro-2-thienyl)methanol as a nucleophile to attack electrophilic drug derivatives, the second was by preparing derivatives of (5-nitro-2-thienyl)methanol for it to act as an electrophile. Alternatively, the thiophene could be introduced without the nitro group, which could be attached later in the synthetic sequence.

The drugs used were the ones selected for the indoledione trigger, namely, prednisolone-21-hemisuccinate, isoquinolin-1-one and its 5-substituted analogues. Aspirin was also chosen as a drug moiety for nitrothiophene triggers and reactions with nifedipine were investigated.

8.1 *PREPARATION OF (5-NITRO-2-THIENYL)METHANOL AND ITS CHLORO DERIVATIVE.*

(5-Nitro-2-thienyl)methanol **119** was obtained by reduction of 5-nitrothiophene-2-carboxaldehyde **118** with sodium borohydride [140]. Different reduction conditions were described in the literature but they mainly used an excess of sodium borohydride in ethanol or methanol[149,150]. Compound **119** was afforded in good yield. The proton NMR spectrum showed the appearance of peaks for H-3 and H-4, observed as doublets at δ 6.94 and δ 7.81, respectively, in the aromatic region. The aliphatic region contained a singlet for the methylene protons at δ 4.88 and another singlet integrating for 1 H at δ 2.03 and disappearing in the presence of D_2O, was identified as the OH peak.

2-Chloromethyl-5-nitrothiophene **120** was synthesised by treatment of **119** with thionyl chloride. The compound was obtained in 52% yield (**Scheme 40**).[148] Proton NMR analysis expectedly did not show any important change in the chemical shifts. TLC analysis confirmed the formation of **120**.



118 **119** **120**

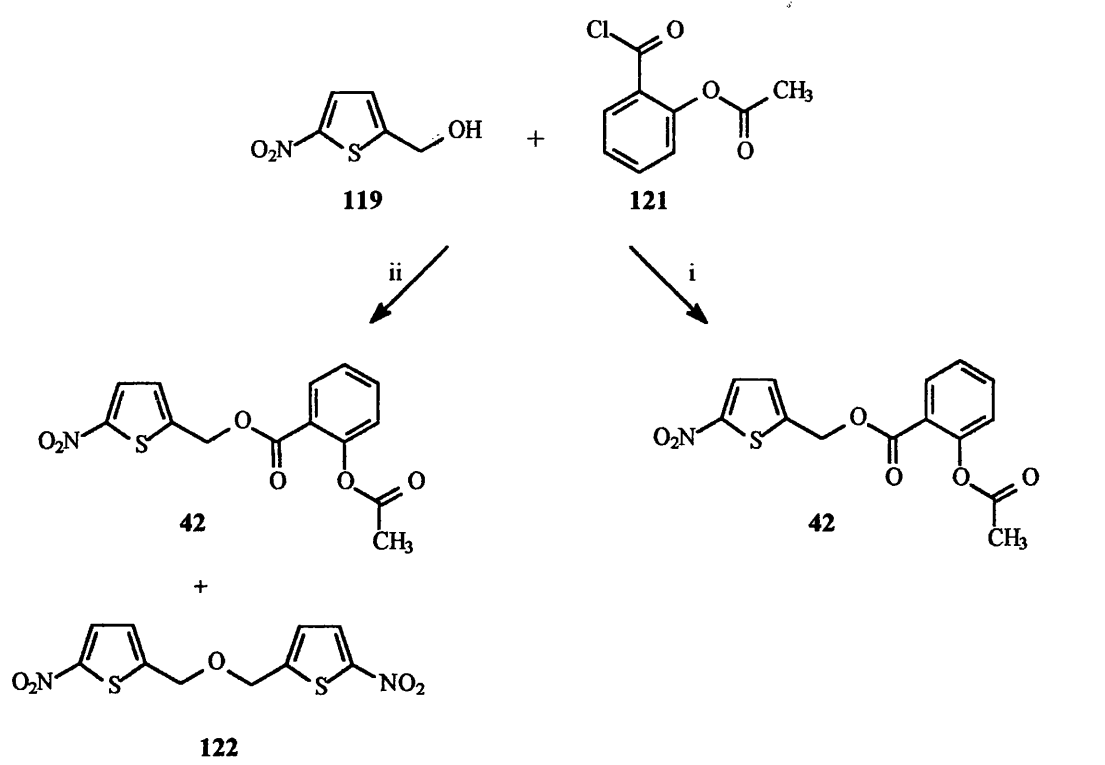
Scheme 40: Synthesis of (5-nitro-2-thienyl)methanol and 2-chloromethyl-5-nitro-thiophene. Reagents: i, $NaBH_4$, MeOH, H_2O; ii, $SOCl_2$.

8.2 *REACTIONS WITH (5-NITRO-2-THIENYL)METHANOL.*

(5-Nitro-2-thienyl)methanol was first used as a nucleophile. Deprotonation of the hydroxyl group with a base would give the alkoxide anion which could then displace a leaving group on a drug derivative.

Trahanovsky *et al*,[151] in their study of the pyrolysis of a series of methyl furfurylbenzoates, reported the synthesis of 3-methylfurfurylbenzoate from benzoyl chloride and 3-methylfurfurylmethanol in the presence of pyridine in ether. Compound **119** was similarly treated with acetylsalicyloyl chloride in the presence of pyridine. TLC analysis indicated the presence of a new substance in the mixture. Proton NMR analysis showed each fraction to contain a mixture of three compounds. Further attempts to purify and isolate the fractions proved to be vain. Distillation led to the decomposition of the thiophene ring.

It was suggested from the complex proton NMR spectra that two of the three compounds present were: the expected target and the dimer of the starting alcohol (**Scheme 41**). The reaction was repeated using triethylamine as a base instead of pyridine. TLC indicated the presence of a newly formed substance. Isolation gave the ester **42** in 35% yield.



Scheme 41: Chemical pathway to the ester **42**. Reagents: i, DCM, Et_3N; ii, DCM, pyridine.

Proton NMR confirmed the formation of **42**. The chemical shifts and splitting pattern allowed to assign the peaks as follows: the doublet of doublets at δ 8.04 was identified as being H-6', further downfield than other protons since it bore the effect of the *ortho* carbonyl group. H-6' was coupled to H-5' and *meta* coupled with H-4' (J = 8.2 and 1.9 Hz). H-4 was observed at δ 7.83 as a doublet (J = 4.3 Hz), deshielded by the vicinal nitro group at position 5 of the thiophene ring. H-5' followed at δ 7.61 resonating as a triplet of doublets due to coupling with H-6' and H-4' (coupling constants J = 7.8 and 1.2 Hz). H-4' at δ 7.33 also resonated as a triplet of doublets due to *ortho* coupling with H-5' and H-3' (same coupling constants as H-5'). H-3' was seen at δ 7.12 as a doublet of doublets, since it was coupled to H-4' and *meta* coupled to H-5' (J = 8.2 and 1.2 Hz). Finally, H-3 came at δ 7.07 as a doublet, coupled to H-4. The singlet expected for the methylene protons, previously at δ 4.88, was observed at δ 5.41 due to the deshielding effect of the ester carboxy group CO_2. The acetate protons were seen as a singlet at δ 2.31.

The structure of this first nitrothiophene-based target contained a weakness, which was the nature of the linkage. The ester bond between the trigger and the drug could be a problem for the release studies; under the model reductive conditions, this bond could easily be hydrolysed to give the thiophene alcohol and salicylic acid. This structural characteristic would also be an indicator, during the release studies, of whether the trigger moiety of the potential prodrug was reductively activated and the drug released by electron transfer, or if the ester linkage was hydrolysed.

8.3 *REACTIONS WITH 2-CHLOROMETHYL-5-NITROTHIOPHENE.*

The use of 2-chloromethyl-5-nitrothiophene **120** involved the action of a nucleophilic species generated from the selected drug moieties to displace the chlorine atom. 2-Chloromethyl-5-nitrothiophene **120** was selected to react, first, with prednisolone. The reaction was carried out in the presence of lithium bis(trimethylsilyl)amide. Increasing reaction time and temperature did not give the expected conjugate. Prednisolone was recovered but no traces of the aromatic were detected by proton NMR analysis. It was speculated that nitrothiophene could be temperature-sensitive and decompose when harsher temperature conditions were applied.

It has been noted in the case of the reaction between 3-(chloromethyl)-1,2-dimethyl-5-methoxyindole-4,7-dione **63** and prednisolone that steric hindrance was an important factor in the non-reactivity of the reagents. The alternative had been to introduce a long carbon chain (a hemisuccinate) as a linker.

A similar reaction of prednisolone hemisuccinate anion was attempted under the conditions described for the formation of **65** but using **120** as the electrophile. The reaction failed to work. The base effect was investigated and sodium hydride was chosen as an alternative base but the reaction proved to be unsuccessful. The thiophene trigger was not always recovered and temperature did not seem to be the only factor.

Nitrothiophenes are known to be prone to undergo ring-opening reactions. Also, the presence of the nitro group at position 5, combined with the electronegativity of the

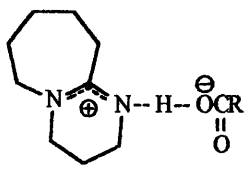
sulfur atom make the methylene protons at position 2 very acidic. The presence of strong bases can lead to the removal of one of those protons rather than to the attack at the carbon atom thus displacing the halogen. After deprotonation, electron-transfer is thought to induce the ring opening[152,153].

1,8-Diazabicyclo[5,4,0]-undec-7-ene (DBU) was also used as an alternative base. Ono *et al*[154] reported a method for the esterification of carboxylic acids with alkyl halides (**Figure 22**). This method was designed for acid- or base-sensitive compounds or sterically hindered acids. Successful esterification of a series of sterically hindered acids, heterocyclic carboxylic acids, thermally unstable acids and N-protected amino acids make this method very interesting and noteworthy.

$$R_1COOH + R_2X \xrightarrow[C_6H_6]{DBU} R_1COOR_2 + DBU.HX$$

Figure 22: Reaction of an acid with an alkyl halide in the presence of DBU following Ono *et al*'s protocol[154].

The principle of this esterification reaction using DBU resembles that of the ion pair extractive alkylation or the crown ether method. In this particular case, the complex is not a pair of free ions but the proton forms a hydrogen-bonded bridge between DBU and the carboxylate (**Figure 23**).



123

Figure 23: DBU-acid complex.

The complex is a strong nucleophile but a weak base; therefore, although the free carboxylate ions could attack both the carbon and the hydrogen of alkyl halides, the

hydrogen-bonded complex attacks preferably the carbon atom. This method is highly selective, compared to the ion pair extraction method.

The method proved to be unsuitable for the proposed type of synthesis. Although prednisolone and prednisolone-21-hemisuccinate were not successfully linked to the nitrothiophene, further investigations were necessary.

Other drug moieties were selected, such as isoquinolinone and nifedipine mentioned earlier. Two model reactions showed precedents in conditions that, so far, had not been used with the nitrothiophene trigger. Benzyl chloride reacted with nifedipine in the presence of lithium bis(trimethylsilyl)amide to give conjugate **103**. Berry *et al*[83] obtained 2-(5-nitrofuran-2-ylmethyl)isoquinolin-1-one by deprotonation of isoquinolin-1-one with sodium hydride and treatment with 2-(tosyloxymethyl)-5-nitrofuran.

Varying the reaction conditions, *i.e.* the base and the solvent together with changing the chlorine atom for a better leaving group might have led to the expected conjugates. Reaction between isoquinolinone or nifedipine with **120** in the presence of lithium bis(trimethylsilyl)amide did not give any positive results.

Reactions of the nitrothiophene alcohol **119** with phosgene gave the chloroformate derivative. Similarly, reactions with ethyl chloroformate and with 1-chloroethyl chloroformate **124** under basic conditions produced the carbonate derivatives. TLC analysis and proton NMR brought evidence for the formation of the carbonates. These compounds were treated with isoquinolinone and nifedipine under the two different reaction conditions previously described but the lack of success led to consideration of another strategy.

8.4 *ALTERNATIVE APPROACHES TO THE 1-(5-NITRO-2-THIENYLMETHYL)-4-(PREDNISOLON-21-YL) BUTANEDIOATE CONJUGATE.*

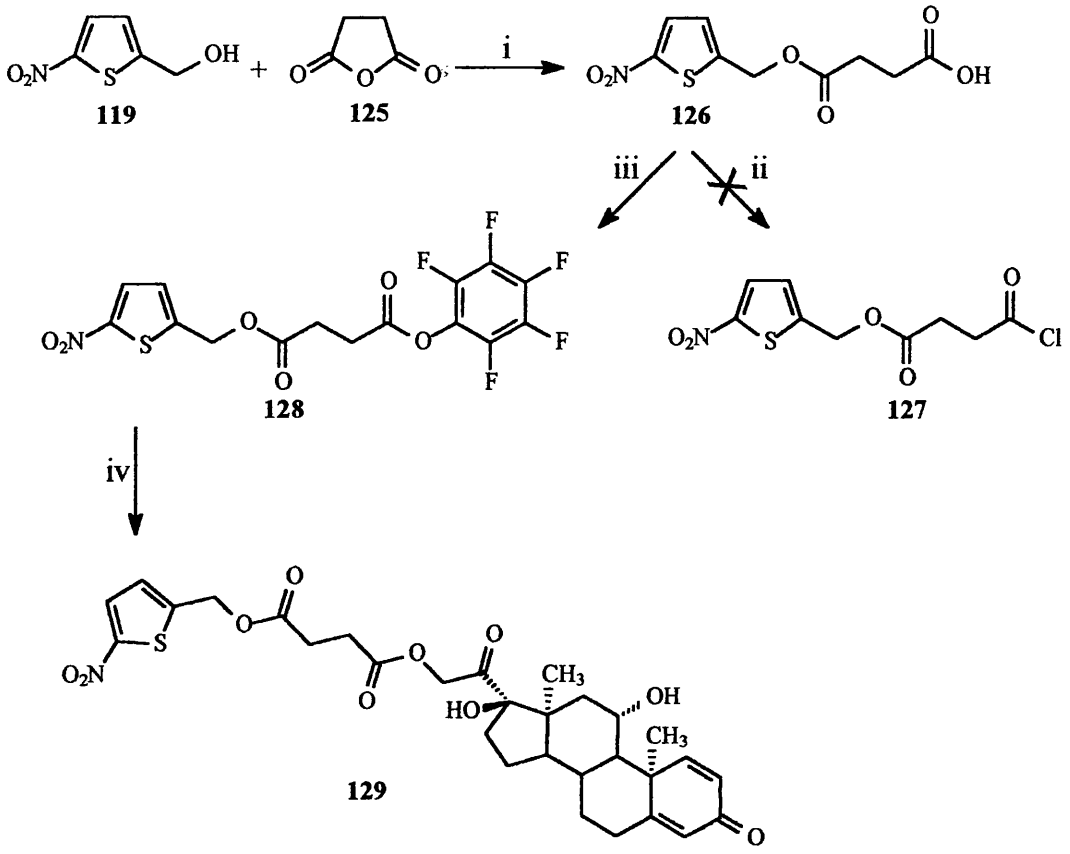
A new strategy was developed based on "building" the linker or spacer chain, that is to say the hemisuccinate subunit, from the nitrothiophene starting material.

Wang and Wong[155] reported the reaction between succinic anhydride and benzyl alcohol in the presence of pyridine and DMAP to yield monobenzyl succinate in 63% yield. The same protocol was applied to **119** and succinic anhydride to yield the acid derivative **126** in 76% yield. ^{1}H NMR analysis showed the thiophene H-4 and H-3 as doublets at δ 7.25 and δ 7.03, respectively, with a coupling constant of 5.0 Hz. The thiophene-CH_2 protons were observed as a single peak at δ 5.27 due to the deshielding effect of the adjacent CO_2 group. The $(CH_2)_2$ came at δ 2.70 as a multiplet, for the overlapping two triplets that were expected.

The next logical step was to produce the acid chloride derivative, making the substance more reactive for nucleophilic attack (**Scheme 42**). Carboxylic acid **126** was treated with oxalyl chloride. The compound was used in the next step with prednisolone under basic conditions but gave no reaction. Further runs of those two steps gave no positive results and mass spectrometry analysis did not show the presence of the acid chloride. An alternative to the acid chloride was used: the pentafluorophenyl active ester[156].

The ester **128** was generated by treatment of **126** with pentafluorophenol and dicyclohexylcarbodiimide. Proton NMR showed no changes in the aromatic region. The methylene protons at position 2 of the thiophene ring were still observed at δ 5.29. Now the strongly electron-deficient pentafluorophenyl group is present at one end of the system, the signals for the two contiguous CH_2 groups were observed at different chemical shifts (δ 3.04 and δ 2.84) with a coupling constant of 6.2 Hz. The signals for the 6 carbons of the pentafluorobenzene ring expected in the ^{13}C NMR spectrum were not observed. The peak height observed for quaternary carbons is usually weak, and the additional ^{13}C-^{19}F coupling splits these signals into complex

multiplets owing to $^{1}J_{C\text{-}F}$, $^{2}J_{C\text{-}F}$ and $^{3}J_{C\text{-}F}$, reducing the peak height further. The mass spectrometric analysis gave further evidence for the formation of the active ester.



Scheme 42: Synthesis of 1-[(5-nitro-2-thienyl)methyl]-4-(prednisolon-21-yl) butanedioate **129**. Reagents: i, pyridine, DMAP; ii, $(COCl)_2$, THF; iii, PFP, DCC, EtOAc; iv, prednisolone, DMF, DMAP, HCl.

Reaction between **128** and prednisolone under basic conditions afforded conjugate **129** in good yield by displacement of the phenolate by the alkoxide ion generated by deprotonation of prednisolone. Although the ^{1}H NMR spectrum showed the characteristic peaks of the nitrothiophene in the aromatic region together with those of prednisolone and, although the integrations were matching, the aliphatic region was too complex, therefore no conclusions could be drawn on whether the conjugate was formed or if the spectrum was showing a one-to-one mixture of the starting materials. Mass spectrometry was the main technique that could be used. Mass and accurate mass determinations for the target compound were obtained, giving the required final evidence.

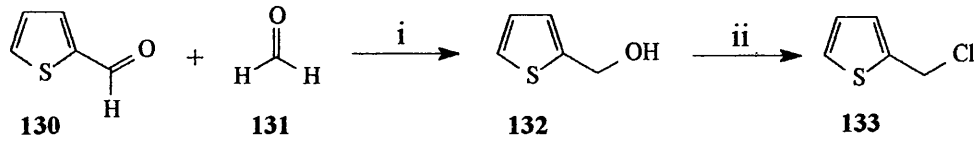
This second target **129** offered an important element of comparison with target **65** for the release studies. The drug and linker unit being the same, the trigger effect could therefore be studied. The choice of this strategy afforded the target with a limited number of steps and offered a reasonable overall yield.

8.5 *NITROTHIENYLMETHYLENE-ISOQUINOLIN-1-ONE CONJUGATES.*

The next type of nitrothiophene-based potential prodrugs that were to be synthesised were bearing the PARP inhibitors. The synthesis of indoledione-based potential prodrugs bearing an isoquinolin-1-one moiety has been described in part **6.3.3**. The synthesis of two potential prodrugs possessing the same drug moiety but having a different trigger counterpart, for which the mechanism of bioreduction is different, offered the possibility to compare two systems for the most efficient drug delivery in future *in vitro* and *in vivo* testing.

Nucleophilic attack from the deprotonated isoquinolinone (or nifedipine) on 2-chloromethyl-5-nitrothiophene or the ethyl and chloroethyl carbonate derivatives gave no sign of reaction. The nitro group at position 5 of the thiophene ring deactivates the ring markedly but is also thought to render the methylene protons at position 2 acidic, as discussed previously. The alternative use of 2-chloromethylthiophene as a trigger unit, followed by nitration of the thiophene ring after the drug linking reaction was investigated.

Thiophene-2-carboxaldehyde **130** was reduced with aqueous formaldehyde and sodium hydroxide, following the method reported by Dunn and Dittmer[157], to give 2-hydroxymethylthiophene **132** in 48% yield, in a process that is related to the Cannizzaro reaction. The 2-chloromethyl derivative **133** was obtained by reaction of **132** with thionyl chloride in the presence of pyridine in 64% yield (**Scheme 43**).



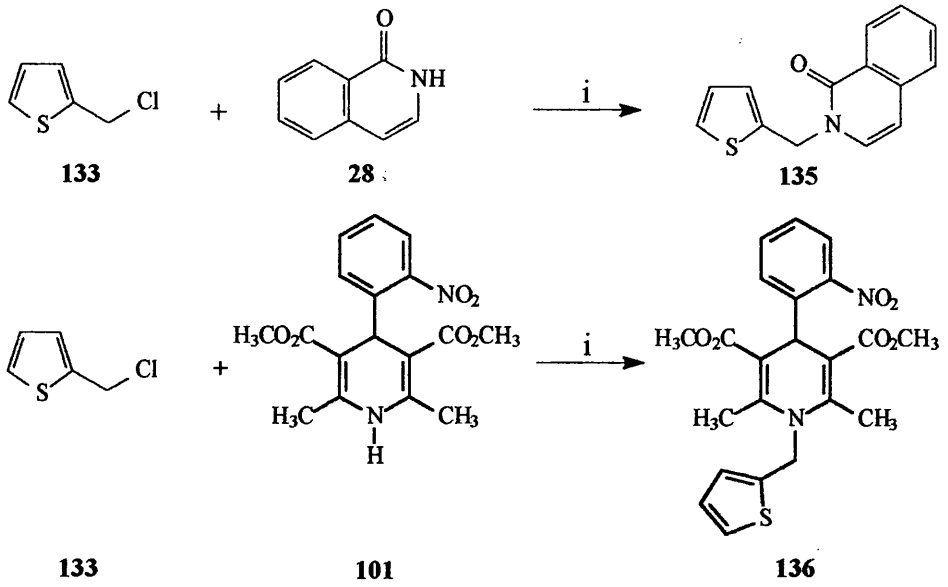
Scheme 43: Synthesis of 2-hydroxymethyl- and 2-chloromethyl-thiophene. Reagents: i, MeOH, NaOH; ii, $SOCl_2$, DCM, pyridine.

The proton NMR spectra of those two compounds were very similar in their splitting pattern and chemical shifts. H-5 was seen as a doublet due to its H-4 neighbour at δ 7.31, δ 7.09 showed H-3 as a doublet as well since it was also coupled to H-4. H-4 arose at δ 6.95 as a doublet of doublets as a consequence of the noted couplings. The methylene protons were seen as a single peak at δ 4.80. In the case of **132**, the peak for OH was observed at δ 2.03 as a singlet.

Following the procedures described earlier, isoquinolin-1-one **28** was deprotonated with lithium bis(trimethylsilyl)amide and the resulting anion reacted with 2-chloro-methylthiophene **133** to give the conjugate **135** in 45% yield. The proton NMR spectrum matched the structure, with shifting of the methylene protons downfield to δ 5.34 and of H-3 in the isoquinolin-1-one to δ 7.50. H-4 of the isoquinoline resonated upfield at δ 6.49, confirming that the thienylmethyl unit was attached at the nitrogen of the isoquinolin-1-one.

A similar reaction was investigated using nifedipine **101** as the drug moiety. The conjugate, **136** was obtained in 10% yield (**Scheme 44**).

The successful course of those reactions provided the evidence that the nitro group was responsible for the lack of reactivity of the thiophene subunit.



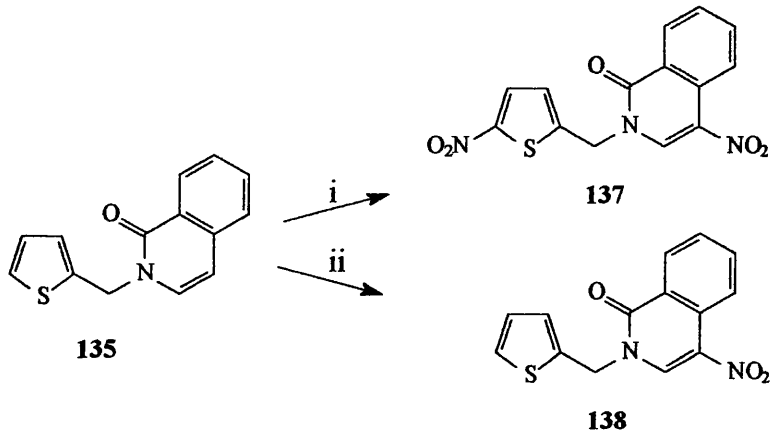
Scheme 44: Synthesis of 2-(2-thienylmethyl)isoquinolin-1-one and dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1-(2-thienylmethyl)-1,4-dihydropyridine-3,5-dicarboxylate. Reagents: i, $LiN(SiMe_3)_2$, THF, Ar.

The yields obtained for those two conjugates were relatively low but the following nitration steps were critical and suspected to be difficult in terms of regioselectivity. For that reason, optimisation of the previous reaction was considered as being of second order and more attention was focused on the nitration.

It is known that electrophilic substitutions are favoured at position 2 of the thiophene nucleus. When a substituent is present at the 2-position, electron-donating substituents will favour nitration at position 5, whereas electron-withdrawing substituents will be equally 5- and 4-directing. Berry *et al*[83] worked on the nitration of 2-(furan-2-ylmethyl)isoquinolin-1-one and reported that mild nitration conditions such as acetyl nitrate, or nitric acid in acetic acid as well as other mild nitrating systems systematically gave the dinitrated product 4-nitro-2-(5-nitrofuran-2-ylmethyl)-isoquinolin-1-one. Studies on substitution of isoquinolinones showed that the principal site of reaction of a range of electrophiles is position 4. However, Berry *et al*[83] exploited the fact that treatment of isoquinolin-1-one with potassium nitrate in concentrated sulfuric acid triggered nitration at positions 5 and 7, due to the deactivation of the heterocyclic ring by protonation. Therefore, treatment of 2-(furan-2-ylmethyl)isoquinolin-1-one in trifluoroacetic acid (TFA) with nitric acid or

copper(II) nitrate at low temperature lead to the selective mononitration of the furan ring at position 5, thus affording their target compound. Traces of the dinitro-compound were detected and the parent isoquinolin-1-one was also isolated resulting from dealkylation under acidic conditions.

Based on those observations and results, **135** was treated with concentrated nitric acid in TFA. Work up after one hour at room temperature gave the dinitro-compound, **137**. Work up after only 30 min at -10°C gave the mono-nitrated derivative **138** (**Scheme 45**).



Scheme 45: Nitration of 2-(2-thienylmethyl)isoquinolin-1-one. Reagents: i, HNO_3, TFA, -10°C, >1h; ii, HNO_3, TFA, - 10 °C, < 1h, HNO_3, AcOH, AcO_2, -10°C or $Cu(NO_3)_2$, TFA.

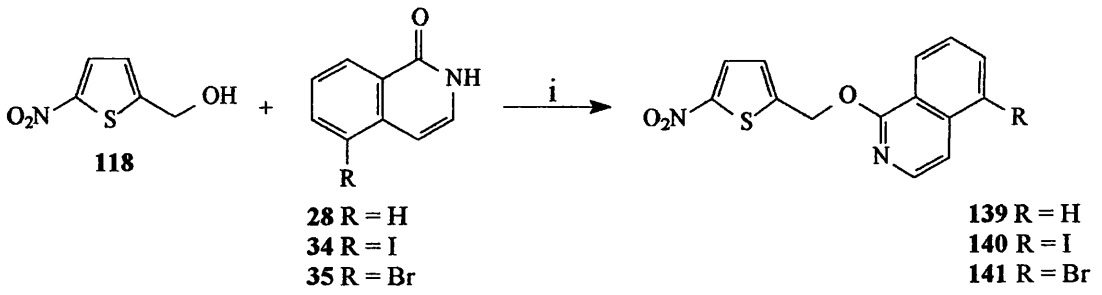
The 4-nitro compound **138** was also obtained in 40% yield by using nitric acid and acetic acid in acetic anhydride at -10°C. Products of dealkylation were observed as minor components of the final mixture. Copper(II) nitrate in TFA gave mono-nitration at position 4 of the isoquinolin-1-one ring. The selective nitration of the isoquinolinone moiety was achieved but none of the systems used afforded selective nitration of the thiophene ring.

Attempts to nitrate the thiophene/nifedipine conjugate **136** under various conditions led either to dealkylation or failure to react.

The next option available after the success with the indoledione trigger was to use the Mitsunobu reaction[124]. 2-Hydroxymethyl-5-nitrothiophene was treated with isoquinolin-1-one **28**, and with 5-iodo- and 5-bromo-isoquinolin-1-ones **34,35** in the presence of the Mitsunobu complex triphenylphosphine / diethyl azodicarboxylate to afford the conjugates **139, 140** and **141**. The yield for those reactions ranged from 30% to 40% (**Scheme 46**).

For each of these compounds, TLC indicated that the mixture was composed of the target compound, an eventual dimer of the parent thiophene, the two starting materials and triphenylphosphine oxide. For the isolated products, proton NMR analysis showed an important downfield shifting of the isoquinoline H-3, H-4 and H-5, as in the case of compound **97**, demonstrating that the nitrothienylmethyl group had attached to the exocyclic oxygen.

Formation of conjugate **139** was proved by the change in chemical shift of the CH_2 group, seen in the NMR spectrum at δ 5.74. The thiophene H-4 was observed as a doublet at δ 7.12, coupled to H-3 also a doublet at δ 7.8, identified by their mutual coupling constant of 4.0 Hz. The isoquinoline H-4 was observed as a doublet at δ 7.3 and had a coupling constant of 6.0 Hz which matched the one of the doublet at δ 8.0 thus identified as H-3. A pseudo triplet followed at δ 7.55 for H-7; H-6 resonated at δ 7.7 also as a pseudo triplet with a coupling constant corresponding to the one of H-7 of 7.7 Hz. H-5 was seen as a doublet at δ 7.75. H-8 was the most deshielded proton on the ring due to the neighbouring carbonyl group and resonated as a doublet at δ 8.25.



Scheme 46: Synthesis of 1-(5-nitro-2-thienylmethoxy)isoquinoline **139** and its 5-iodo and 5-bromo analogues. Reagent: i, PPh_3, DEAD, THF.

The deshielded character of the CH_2 group in the molecule observed in the ^1H NMR and the comparison between the ^{13}C NMR shift of the CH_2 group in **140** and in the furan analogue of **139** reported by Berry *et al* [83] suggested that attachment took place at the oxygen rather than at the nitrogen atom of the isoquinolinone as observed in the case of compounds **93** and **99**. No concomitant N-alkylation was observed for any of the compounds. The presence or absence of substituents at position 5 of the isoquinolinone ring did not affect the outcome of the reactions.

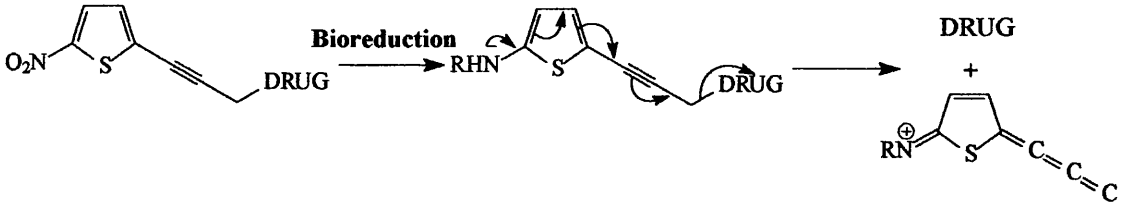
Proton NMR analyses for the following 5-substituted analogues were slightly different. Proton shifts for H-8, the thiophene H-3 and H-4 and the methylene protons were similar to the ones observed for the first conjugate. For both analogues, H-6 and H-3 resonated at similar chemical shifts. In the case of **140**, H-6 was observed after H-8 at δ 8.22 followed by H-3 at δ 8.10. In the case of the bromo derivative, H-3 was seen first at δ 8.12 followed by H-6 at δ 7.97. This could be a consequence of the different negative inductive effect applied by iodine compared to bromine on the ring. H-4 was also noticed in both conjugates to appear downfield compared to H-7, which was the opposite of what was observed in the parent drug moiety and in the first conjugate. In **140** H-4 was seen at δ 7.49 followed by the pseudo triplet of H-7 at δ 7.27. Similarly for **141**, the doublet of H-4 came at δ 7.65 and the pseudo triplet of H-7 came at δ 7.42.

The Mitsunobu method enabled the synthesis of three conjugates, bringing the total number of nitrothiophene-based conjugates to five.

It was demonstrated that 2-hydroxymethyl-5-nitrothiophene **119** could be used to generate the nucleophilic alkoxide anion and attack an electrophilic centre on a drug derivative to yield the corresponding conjugate. However, as an electrophile, the 5-nitro derivative **119** did not react as expected due to the electron-withdrawing effect of the nitro group, presumably rendering the methylene protons too acidic. The Mitsunobu alternative was used to overcome this problem. The apparent selective formation of the ether raises more questions concerning the mechanism leading to the formation of the potential prodrugs.

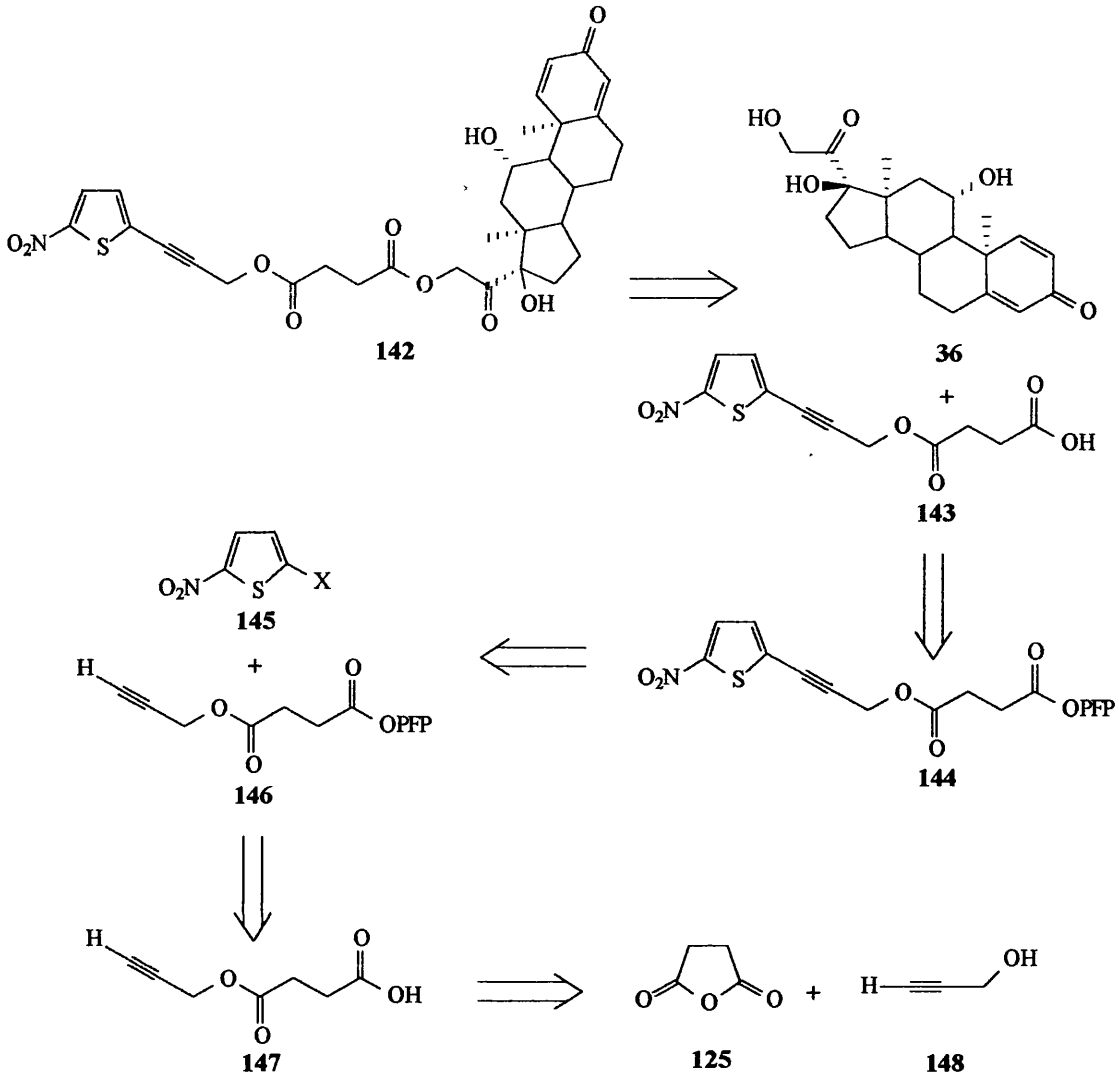
8.6 *ATTEMPTED SYNTHESIS OF 1-[3-(5-NITRO-2-THIENYL)-2-PROPYN-1-YL]-4-(PREDNISOLON-21-YL) BUTANEDIOATE 142.*

In order to study as many dimensions of the problem as possible, it was planned to synthesise an analogue of **129**, namely 1-[3-(5-nitro-2-thienyl)-2-propyn-1-yl]-4-(prednisolon-21-yl) butanedioate **142**, by introducing a triple bond conjugated to the thiophene ring. By increasing the conjugated system, especially by introducing a multiple bond, a another parameter was introduced that was expected to influence the release efficiency, by analogy with the 3-alkynylindolediones.



Scheme 47: Proposed mechanism for the bioreductively triggered release of drugs from 5-nitro-2-thienylpropynyl potential prodrugs. [139]

Retrosynthetic analysis allowed identification of the potential starting materials and synthetic steps.



Scheme 48: Retrosynthetic analysis of synthetic target **142**.

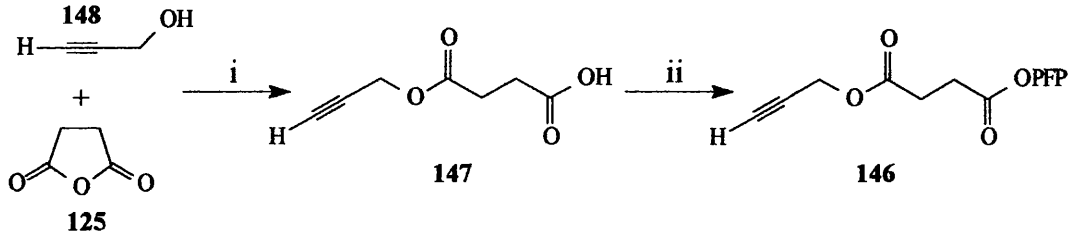
The major step that is highlighted by this retrosynthetic study is the coupling of the terminal alkyne **146** to the 2-substituted-5-nitrothiophene (**Scheme 48**). The creation of such a carbon-carbon bond has already been studied in the present work, in the case of the indole trigger.

Palladium-catalysed coupling reactions on thiophenes have previously been reported; Rossi *et al*[158,159] , in their work on naturally occurring acetylenic thiophenes, used 5-substituted-2-iodothiophene derivatives in the presence of tetrakis(triphenyl-phosphine)palladium, CuI as catalyst and 2.5 N sodium hydroxide as a base. Further work on the synthesis of acetylenic thiophenes published by van den Hoven and

Alper[160] described a similar method for coupling reaction between 2-iodothiophene and a series of acetylenes bearing different functionalities (including simple terminal alkynes, propargyl ethers, alcohols, or esters, phenylacetylenes and enynes) in the presence of $Pd(PPh_3)_4$ and CuI. Relatively good yields were reported, ranging from 65% to 90%. Wu *et al*[161], in their study of push-pull type chromophores prepared from nitrothiophene-induced Michael-type reaction of alkynes reported the synthesis of nitrothienylalkynes from terminal alkynes and 2-bromo-5-nitrothiophene *via* Sonogashira coupling catalysed by bis(triphenylphosphine)palladium dichloride and copper(I) iodide.

Those examples seemed to offer the possibility to couple either the 2-halothiophene or the 5-nitro-2-halothiophene with the terminal alkyne chosen.

The synthesis of the required acetylene was based on the method used for compound **128** (**Scheme 42**). Reaction between propargyl alcohol and succinic anhydride under basic conditions afforded acid **147** in 64% yield (**Scheme 49**). Proton NMR data matched the structure of the compound formed: the methylene protons of the propargyl moiety were shifted to δ 4.71, due to the carboxy group. The two CH_2 groups of the succinic subunit were observed as a multiplet at δ 2.88 due to the overlapping of the two triplets expected. Finally, the methine proton was observed at δ 2.49. The active ester of **147** was prepared following the protocol described for **128**, affording **146** in 100% conversion.

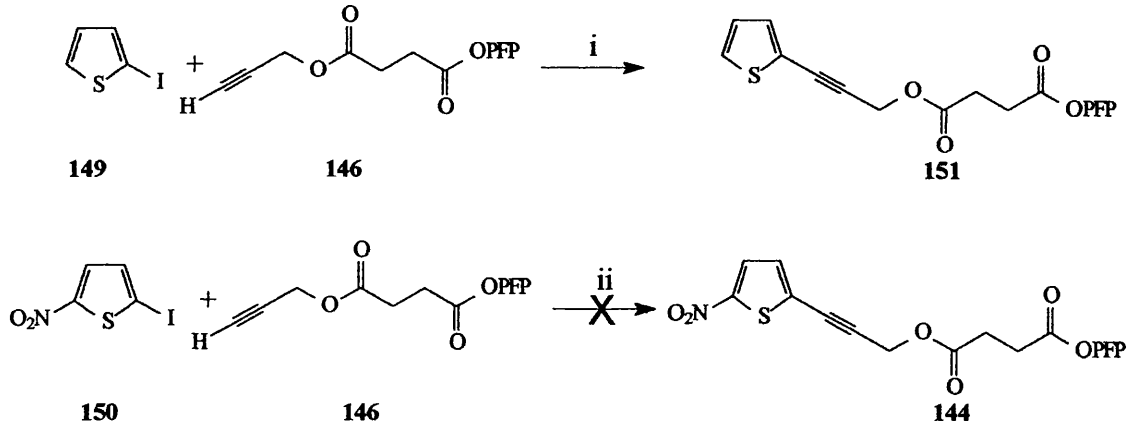


Scheme 49: Synthesis of 4-oxo-4-(prop-2-ynyloxy)butanoic acid **147** and 1-(pentafluorophenyl)-4-(prop-2-ynyl) butanedioate **146**. Reagents: i, pyridine, DMAP; ii, PFP, DCC, EtOAc.

The proton NMR showed no change in chemical shift for the CH_2 group α to the triple bond and for the methine proton but the multiplet observed for the two CH_2 groups changed into two distinct triplets at δ 2.80 and δ 3.03, respectively.

2-Iodothiophene **149** was commercially available but 2-iodo-5-nitro-thiophene **150** was synthesised following the method used by D'Auria *et al*[162]. 2-Iodothiophene **149** was nitrated with acetic anhydride and nitric acid at low temperature to give derivative **150** in 70% yield.

Coupling reactions between **149** and **150** (thiophene and nitrothiophene) and **146** (alkyne) were carried out in parallel, following the general protocol described by van den Hoven and Alper[160] using $Pd(PPh_3)_4$ as the palladium catalyst (**Scheme 50**).



Scheme 50: Coupling reaction of 2-iodothiophene and its 5-nitro analogue to 1-(pentafluorophenyl)-4-(prop-2-ynyl) butanedioate. Reagents: i, $Pd(PPh_3)_4$, CuI, Ar, Et_3N, C_6H_6.

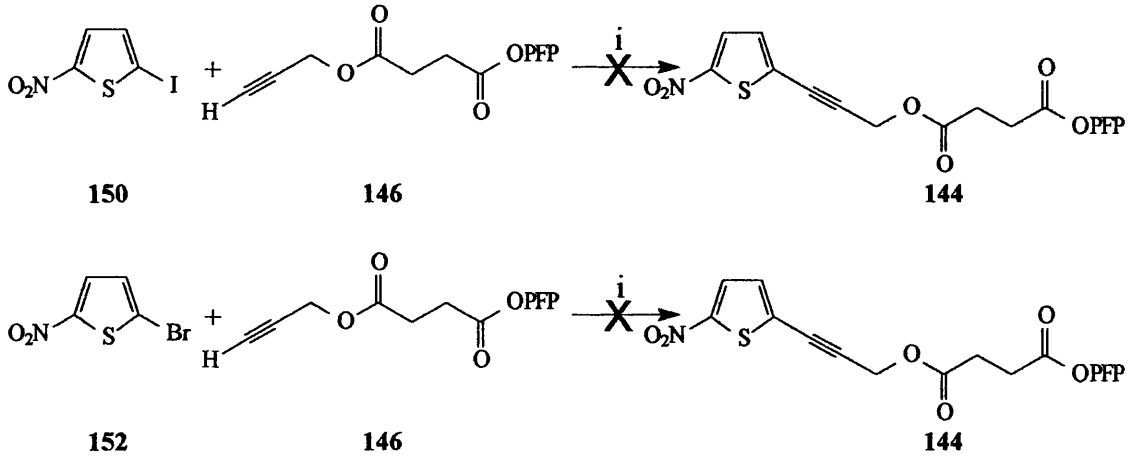
Only the non-nitrated thiophene **149** coupled successfully to the alkyne affording **151** in 52% yield.

Nitration of **151** was attempted but, even under mild conditions (nitric acid in acetic acid or trifluoroacetic acid at low temperature or copper(II) nitrate in trifluoroacetic acid), gave a complicated mixture in which could be identified the 5-nitrated

thiophene derivative but the ester bond to the succinic subunit seemed to be hydrolysed.

The nature of the catalyst and of the halogen on the thiophene are known to be factors influencing the reaction. To investigate their effect on this coupling reaction, the method published by Wu *et al*[161] was used. 2-Bromo-5-nitrothiophene was prepared according to the method used for 2-iodothiophene and gave the derivative in 60% yield.

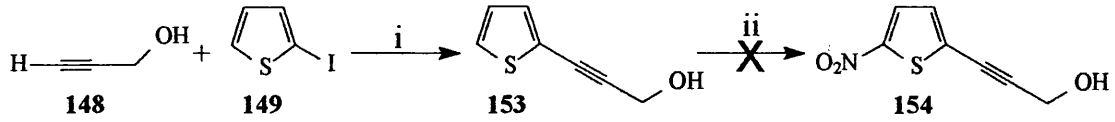
Both 2-iodo- and 2-bromo-5-nitrothiophene **150** and **152** were treated, in parallel, with acetylene **146**, in the presence of $Pd(PPh_3)_2Cl_2$. No sign of reaction was observed (**Scheme 51**).



Scheme 51: Coupling reaction following the Wu method[161]. Reagents: i, $Pd(PPh_3)_2Cl_2$, CuI, Ar, $^iPr_2NH$.

The last approach for the synthesis of the target proposed was to exploit the fact that coupling of the alkyne to 2-iodothiophene was successful, it was then proposed to couple propargyl alcohol to 2-iodothiophene, nitrate the resulting compound and react the nitrated derivative with succinic anhydride, followed by the expected preparation of the active ester and reaction with prednisolone (**Scheme 52**).

The coupling reaction was carried out under the conditions previously described for the non-nitrated iodo-compound.



148 **149** **153** **154**

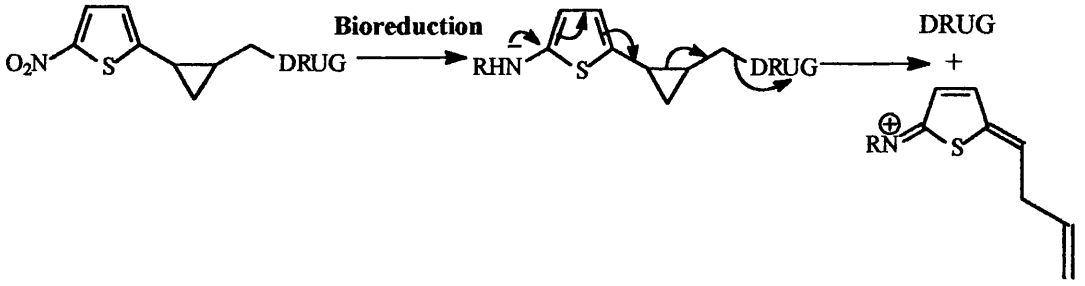
Scheme 52: Coupling reaction of 2-iodothiophene with propargyl alcohol and nitration of the product. Reagents: i, $Pd(pph_3)_4$, CuI, Ar, Et_3N, C_6H_6, ii, HNO_3, TFA, - 10 °C.

153 was obtained in 67% yield. Nitration of **153** in TFA and nitric acid did not give the nitrated compound **154**. Stronger conditions led to the decomposition of the ring.

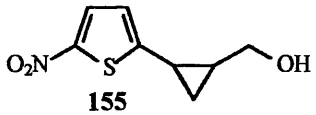
Although the coupling reaction gave the expected compound, the nitration step was a critical reaction and no appropriate system was found to overcome the problem so far.

9. SYNTHESIS OF NITROTHIOPHENE-BASED NEW TRIGGERS.

The synthesis of new thiophene triggers was also considered. The role of these new triggers was to compare and optimise the ease of reduction and release the drug non-reversibly. The introduction of a cyclopropane ring α to the thiophene ring was another idea to modify the thiophene trigger in an interesting way (**Figure 24**). Reductive activation of the proposed trigger and electron transfer would result in the ring opening of the cyclopropane leading to the eventual *irreversible* delivery of the drug moiety (**Scheme 53**).



Scheme 53: Proposed mechanism for the bioreductively triggered release of drugs from 5-nitro-2-thienylcyclopropylmethyl potential prodrugs.

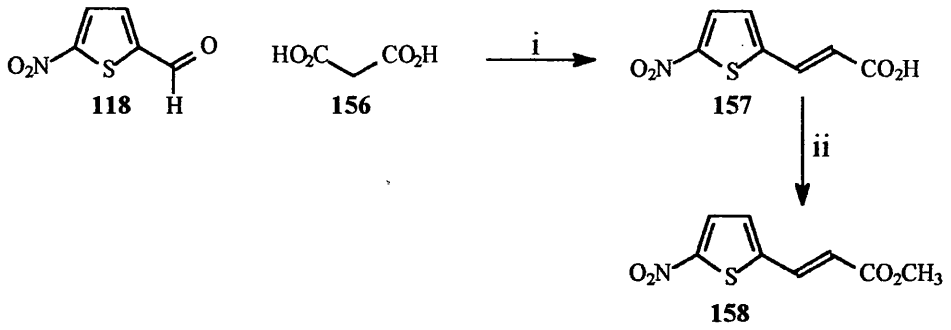


155

Figure 24: Structure of the proposed trigger.

Retrosynthetic analysis indicated a possible chemical pathway to the thienylcyclo-propane **155** *via* the Knoevenagel condensation reaction of 5-nitro-2-thiophene-carboxaldehyde and malonic acid reported by King and Nord[163].

The condensation reaction between aldehyde **118** and acid **156**, in the presence of pyridine gave acrylic acid **157** in high yield. Its methyl ester **158**, was obtained by treatment with methanol in the presence of sulfuric acid. Proton NMR data matched the compound's structure (**Scheme 54**).



Scheme 54: Synthesis of 5-nitro-2-thienylpropenoic acid and its methyl ester. Reagent: i, pyridine, piperidine; ii, MeOH, H_2SO_4.

Cyclopropanation could be tried directly on this last intermediate, but it could also be used to produce a simpler trigger. The double bond is conjugated to the ring similarly to the triple bond in the proposed target **142**. By reducing either the acid or the ester to the alcohol, a simple model of nitrothiophene trigger with a conjugated multiple bond would be obtained (**Figure 25**).

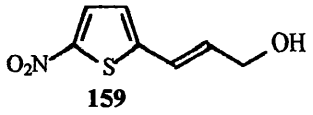


Figure 25: Structure of the proposed trigger 3-(5-nitro-2-thienyl)prop-2-enol **159**.

Pontikis *et al*[164] converted a series of heterocyclic acrylic esters into the allylic alcohols and finally to their acetates. The starting acrylic acids were treated with ethyl chloroformate in the presence of triethylamine in THF. The resulting ethyl esters were then treated with sodium borohydride in THF and methanol at low temperature to afford the corresponding allylic alcohol.

5-Nitro-2-thienyl-3-propenoic acid **157** was converted into its ethyl ester **158**, according to the method described, in 80% yield. However, the attempted reduction with sodium borohydride was unsuccessful. Reduction under harsher conditions, such as use of lithium aluminium hydride, resulted in the decomposition of the starting ester.

D'Auria and Ferri[165] used thiophenepropenoic acid to produce the allyl alcohol, but without the nitro group at position 5. Lithium aluminium hydride was used in THF at low temperature. The multiple attempts resulted in decomposition of the starting material. More attention was then given to the cyclopropyl trigger.

The formation of cyclopropane rings from vinylthiophenes can be achieved by different methods. Simmons and co-workers[166,167] reported a method using diiodomethane and a zinc-copper couple (Simmons-Smith reaction).

LeGoff[168] suggested a method for the preparation of the zinc-copper couple (as a granular form or as a dust) before the reaction of cyclopropanation. Rawson *et al*[169] proposed a one-pot reaction using zinc dust and a cuprous halide. The protocol involved the preparation of a mixture of zinc dust and cuprous chloride in ether heated under reflux and nitrogen atmosphere. The olefin was then added followed by diiodomethane and the mixture was boiled for 24 h.

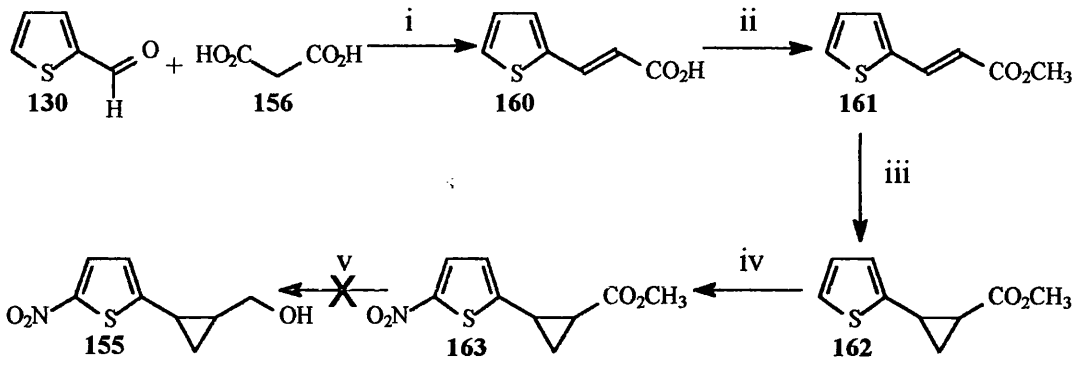
Paulissen *et al*[170] reported a palladium-catalysed cyclopropanation of olefins using palladium acetate in the presence of ethyl diazoacetate or diazomethane. The model reactions were carried out under mild thermal conditions and were thought to proceed *via* a carbene-metal-olefin complex.

The method of Simmons[166,167] was studied first. Reactions were carried out using the acid **157** and the ester **158**. The starting material was recovered in each case without any sign of reaction. To prove that the electron-withdrawing effect of the nitro group had no effect on the reactivity of the alkene, the same reactions were carried out using 2-thienylacrylic acid **160** and the corresponding ester **161** but no cyclopropanation occurred.

Paulissen's[170] method was then used. The four starting materials, that is to say the nitrated and non nitrated thienyl acrylic acids **157** and **160** and their methyl esters **158** and **161** were investigated in parallel. Treatment of these alkenes with ethyl diazoacetate in the presence of palladium acetate as the catalyst failed to afford cyclopropanes.

Vallgarda *et al*[171] synthesised and studied a range of *trans*-2-arylcyclopropylamine derivatives from substituted arylpropenoate esters; methyl 2-thienylpropenoate was converted to methyl *trans*-2-thienylcyclopropane-1-carboxylate by the action of palladium acetate on a saturated ethereal diazomethane solution. The relative concentration of palladium catalyst was of 0.005 equivalents strictly, as higher concentrations led to the precipitation of palladium (0) with consequent termination of the reaction.

Application of this method to **158** and **161** required the preliminary generation of diazomethane. Diazomethane was prepared by treatment of Diazald® with potassium hydroxide. Reaction of **158** and **161** in the presence of $Pd(OAc)_2$ (10 mol%) and diazomethane gave **162** in high yield. The corresponding nitro derivative was not obtained and no trace of the starting material was detected by proton NMR analysis. ^1H NMR analysis of compound **162** showed a complicated splitting pattern in the aliphatic region. The four protons of the cyclopropyl ring were split into multiplets. The CH_2 protons were, as expected, inequivalent. The three-membered ring conformation involves a highly strained structure causing the protons to resonate upfield from a usual alkane position. Each proton of the three-membered ring was influenced by three inequivalent neighbours, explaining the splitting pattern observed. The four multiplets were observed at δ 2.7, δ 1.9, δ 1.6 and δ 1.3. The methoxy protons were seen at δ 3.7 as a single peak. The aromatic protons of the thiophene ring were observed as three doublets of doublets at δ 7.09 for H-5, δ 6.89 for H-4 and δ 6.81 for H-3 suggesting ^4J coupling between H-3 and H-5. The coupling constants accompanying the signal of those protons were respectively 3.5 and 1.3 Hz and 5.3 and 1.3 Hz respectively, proving the existence of ^4J coupling.



Scheme 55: synthesis of methyl 2-(5-nitro-2-thienyl)cyclopropane-1-carboxylate. Reagents: i, pyridine, piperidine; ii, MeOH, H_2SO_4; iii, CH_2N_2, ether, $Pd(OAc)_2$; iv, TFA, HNO_3; v, DIBAL-H or $LiAlH_4$.

Regioselective nitration of **162** led to the next intermediate **163** (**Scheme 55**). The final step towards the new trigger was the reduction of the ester derivative to the alcohol. Reduction of the ester to the alcohol required methods involving diisobutylaluminium hydride or lithium aluminium hydride. Both reagents were investigated and gave disappointing results: DIBAL-H gave no reaction, the starting cyclopropyl ester was recovered from each attempt and $LiAlH_4$ was suspected to trigger the cyclopropane ring-opening. The proton NMR data obtained after reaction were unclear and did not allow the proper identification of the compounds obtained. Further studies are required on that final step that would lead to a new interesting trigger.

In addition to the nitrothiophene-based targets, the design of two new triggers was proposed, but although the synthesis of the compounds was not achieved, some interesting aspects of the thiophene chemistry were highlighted.

10. RELEASE STUDIES

10.1 *INDOLEDIONE-BASED POTENTIAL PRODRUGS*

A number of method have been reported for mimicking the reductive activation of quinone systems: *e.g.* electrochemistry, catalytic hydrogenation, Cr(II), sodium dithionite. Radiolytic reduction methods have also been reported by Naylor, Everett and co-workers[50-52]. They allowed the quantification of the efficiency of the leaving group elimination by studying the kinetic properties of the radical intermediates generated (semiquinone radical reactivity towards oxygen, effect of oxygen on leaving group release).

Sodium dithionite had been used to activate mitomycin C and proved to be a convenient method that was therefore used by Naylor *et al*[51]. The reduction experiment was carried out on a series of 1,2-dimethyl-4,7-dioxo-5-methoxyindole-3-methylene potential prodrugs and models, using sodium dithionite as a reducing agent in the presence of a thiol to trap the electrophilic iminium intermediate. The role of the thiol was determined by its nucleophilic properties in mimicking the scavenging cellular thiols such as glutathione. The series of indolequinones was reduced with a large excess of sodium dithionite and five equivalents of thiol, in aqueous degassed THF. Naylor *et al* [151]reported the elimination of the (model) drugs from position 3 by detecting both the (model) drugs and the thiol-trapped indolediones.

Tin(II) chloride has been used in the case of the nitroaromatic bioreductive triggers and proved to be a selective reducing agent of the nitro group[80,83]. The use of tin(II) chloride with selected indolequinone derivatives synthesised in the present study led to the release of the drug counterpart. TLC analysis was first used to prove the efficiency of the method. HPLC analysis was then required to bring further evidence for the reductive activation of the potential prodrug and subsequent release of the drug. The wavelength of maximum absorption was determined for each target to allow efficient UV detection.

Treatment of the potential prodrug **97** with $SnCl_2$ in methanol gave two fully resolved peaks with retention times (Rt) of 4.3 and 4.9 min. HPLC analysis of the starting

indole **11** in the same conditions gave a peak with Rt= 4.6 min. The isoquinolin-1-one counterpart **28** gave a peak at Rt= 4.9 min which matched the second peak observed after addition of the reductive agent and the results described by Parveen *et al*[80] after reduction of their nitroimidazole-based potential prodrug (identical reduction system, HPLC instrument and operator). In the same HPLC system, the parent potential prodrug **97** was detected as a single peak with a retention time of 6.0 min, which was not observed after addition of tin(II) chloride. (**Appendix 1A**)

The reductively triggered release of drugs from their indoledione potential prodrugs was then monitored directly by proton NMR. The potential prodrugs were dissolved in deuterated chloroform due to their limited solubility in deuterated methanol. A tin(II) chloride solution was prepared in deuterated methanol. A proton NMR spectrum of each sample was run, a known quantity of $SnCl_2$ in deuterated methanol was then added and the proton NMR of the new mixture was run immediately. Further additions of the reducing agent were carried out in the same conditions.

Compound **96** (**Appendix 2**) was the first potential prodrug of the indoledione series to be studied by HPLC and was therefore the first one subjected to the NMR study as an element of comparison. Following the addition of $SnCl_2$, changes in the chemical shifts of some of the peaks were observed. 3-H and 5-H were shifted upfield from δ 8.0 and δ 7.7, respectively, to δ 7.37 for 5-H and δ 6.88 for 3-H. Similarly 4-H moved from δ 7.2 to δ 6.4 after reduction. 8-H remained unchanged, 6-H and 7-H were slightly shifted upfield by approximately 0.1 ppm. The methylene group at position 3 of the indole ring, which was also the linking point between the 2 subunits, shifted from δ 5.72 to δ 4.4, which was the final evidence that release of the drug moiety occurred. The chemical shifts observed after reduction corresponded to the ones of an equimolar mixture of isoquinolin-1-one **28** and 1,2-dimethyl-3-(hydroxymethyl)-5-methoxyindole-4,7-dione **11**. These results suggest that the electrophilic species produced after reduction and drug release was quenched by water and reoxidised to the quinone. (**Appendices 3 and 4**)

The methyl, N-methyl and methoxy protons of the indole showed a slight difference in chemical shift compared to the starting material and potential prodrug which was attributed to the effect of the solvent mixture (deuterated chloroform and methanol). Evidence for that phenomenon was obtained by running a proton NMR spectrum of a mixture of **28** and **11** in the same solvent mixture.

It was postulated that tin(II) chloride, being a Lewis acid, may have catalysed the cleavage of the carbon-nitrogen bond instead of the expected quinone reduction triggering the release of the drug moiety. As a control, a similar NMR-monitored experiment was then carried out using $SnCl_4$, which is a stronger Lewis acid but exhibits no reductive properties. No cleavage was observed.

The generation of paramagnetic compounds, possessing an unpaired electron (*i.e.* radicals) causes peak broadening and changes in chemical shifts in proton NMR spectra. No such effects were observed at any time during the release studies.

A preliminary experiment carried out on **97** contaminated with diethyl hydrazine-1,2-dicarboxylate showed complete release of the drug after the addition of 0.1 equivalent of $SnCl_2$. In contrast, the pure potential prodrug **97** did undergo stoichiometric reductively triggered release; spectra taken after the addition of 0.1, 0.2, 0.5 and 1 equivalents showed the appropriate molar ratios of **97** and the products **11** and **28**. These observations are consistent with a stoichiometric reduction and subsequent drug release caused by the Sn(II) in the latter experiment. In the former experiment, it is likely that the Sn(IV) produced in the reaction is reduced back to Sn(II) by the diethyl hydrazine-1,2-dicarboxylate, making the process *apparently* catalytic in tin. Tin(IV) has not previously been reported to oxidise hydrazine-1,2-dicarboxylates but this oxidation has been carried out with Pb(IV)[172].

The 5-substituted analogues of **97**, namely **39**, **40** and **99** were treated with $SnCl_2$ under the same conditions. The results observed were somewhat different.

Addition of one equivalent $SnCl_2$ to the N-linked 5-bromoisoquinolin-1-one **40** (**Appendix 5**) did not show any effects on the compound. Further addition of up to five equivalents did not trigger the release of the 5-bromoisoquinolinone. After 24 h,

two sets of new peaks were observed in a 1:10 ratio with the target peaks. The number of peaks and the splitting pattern suggested that they were isoquinolinones; No other peaks were detected in the aliphatic region. Speculations were made on the possibility that, under reductive conditions, the bromine atom at position 5 could be cleaved reductively (**Appendices 6 and 7**). Parveen *et al*[80] reported that, under other reductive conditions, (sodium borohydride and palladium on charcoal) reductive debromination of the 5-bromoisoquinolinone occurred after its release from the initial nitroimidazolylmethyl potential prodrug.

The N-linked 5-iodo analogue **39**, when submitted to the same reductive conditions, also did not show any signs of release of the 5-iodoisoquinolin-1-one **34** or of the free indoledione-methanol **11** (**Appendix 8**).

In contrast, when the O-linked 5-iodoisoquinoline **99** (**Appendix 9**) was subjected to similar reductive conditions and monitored by proton NMR, the addition of one equivalent of $SnCl_2$ led to the complete release of the drug. Significant shifting of 3-H and 4-H was observed from δ 8.08 to δ 7.12 and δ 7.40 to δ 6.76, respectively. The 3-CH_2 protons of the indole shifted from δ 5.71 to δ 4.52, as observed in the case of **97**, confirming complete release of the drug **34** and regeneration of the indoledione **11** (**Appendix 10**). This NMR approach to studying release of drugs and other moieties from their 4,7-dioxoindole-3-methylene derivatives is the first report of evaluation of potential bioreductively activated prodrugs in this way.

Subjection of the indoledione-prednisolone potential prodrug **65** (containing the succinate linker) (**Appendix 11**) to the same conditions gave surprising results. The side-reaction, hydrolytic/methanolytic cleavage of the ester, was not observed by the proton NMR spectrum. Moreover, the overall chemical shifts were not modified when either the tin(II) chloride or sodium dithionite reductive system was used, suggesting that no release of the drug moiety occurred. (**Appendices 12 and 13**).

Treatment of analogous pentamethylmelamine potential prodrug **94** (**Appendix 14**) with excess $SnCl_2$ gave complex and largely uninterpretable results. The spectrum obtained after addition of tin(II) chloride in deuterated methanol was composed of

broad peaks masking all signals. The addition of deuterium oxide allowed the partial reduction of some of the broad peaks, presumably due to complexes formed between the tin and the drug moieties; the addition of deuterated water allowed the transfer of the tin and of a fraction of the methanol in the aqueous phase. Although the peaks of the starting material could still be identified, a second set of peaks corresponding to the free drug were also identified together with H-6 and CH_2 from the indoledione **11** at δ 5.5 and δ 4.60, respectively. Some of the drug moiety appeared to be release from **94**. However, the presence of remaining broad peaks together with only an apparent partial release of the pentamethylmelamine in the presence of an excess of reductant do not allow one to draw conclusions (**Appendices 15 and 16**). The deuterated analogue **96** (**Appendix 17**) gave similar results (**Appendix 18**).

Some general trends could be drawn from the release studies of the series of indoledione-based potential prodrugs. Firstly, the observation of release from the O-(indolylmethoxy)isoquinolines but no release from the N-(indolylmethyl)isoquinolin-1-ones is consistent with the potentially greater leaving group ability of the oxyanion, compared to the nitrogen anion. When the indoledione is reduced to the corresponding 4,7-dihydroxyindole, the latter has only two possible fates: expulsion of the drug or reoxidation back to the indoledione potential prodrug. Thus the drug moiety has to be a sufficiently good leaving group to be expelled rapidly during the lifetime of the reduced indole unit. Clearly, the carboxylate of prednisolone-21-hemisuccinate, although predicted to be a better leaving group than the isoquinolinone nitrogen anions, is still not sufficiently powerful. Naylor *et al* [51] report efficient expulsion of a range of different model leaving groups (carboxylates, phenolates and hydroxide) from this indoledione trigger but their experiments were conducted using ten equivalents of sodium dithionite as reductant and in the presence of ethyl xanthate as a trapping agent, conditions which are much more vigorous that those in the present study. The larger excess of the more powerful reductant would prolong the effective lifetime of the 4,7-dihydroxyindole initial intermediate, whereas the thiolate-trapping agent would make the expulsion of the leaving group appear to be effectively irreversible.

10.2 *NITROTHIOPHENE-BASED POTENTIAL PRODRUGS*

The methods available to reduce selectively an aromatic / heteroaromatic nitro group are numerous. Everett *et al*[82] reported the reduction of the 2-nitroimidazole to the nitro radical anion using pulse radiolysis and γ-radiolysis. Other methods include catalysed hydrogenation, although this has been reported to trigger hydrogenolysis at the "benzylic" position, that is to say the linking $ArCH_2$-O or $ArCH_2N$ bonds.

Sodium borohydride in the presence of palladium on charcoal, but also tin(II) chloride and titanium trichloride have been used successfully to reduce aromatic nitro groups selectively and thus to trigger release of the drug moieties. Berry *et al*[83] reported the release of the drug counterpart from nitrofuran-based prodrugs following reduction to the aminofuran intermediate using sodium borohydride in the presence of palladium on and using tin(II) chloride. Parveen *et al*[80] used zinc dust in the presence of ammonium chloride in aqueous methanol, the sodium borohydride method described by Berry *et al*[83], and tin(II) chloride in methanol to achieve the reduction of a nitroimidazole-based prodrug.

The effects of reduction on nitrothiophene-based potential prodrug series were initially studied by HPLC. Injection of a solution of the nitrothienylmethyl aspirin compound **42** gave a single peak at Rt = 3.4 min. The nitrothiophenemethanol **119** and the salicyloyl counterpart (aspirin) under the same conditions were detected at Rt = 3.1 and 3.9 min, respectively. Potential prodrug **42** was then treated with zinc powder in the presence of NH_4Cl and the mixture was analysed at regular time intervals. After 1 min, two peaks were observed at Rt = 3.1 and 3.7 min. These results suggested rapid ester hydrolysis, rather than reduction of the nitrothiophene.(**Appendix 1B**)

The analogous nitrothienylmethyl prednisolone hemisuccinate compound **129** was subjected to treatment with $SnCl_2$ in methanol. No reaction was observed at 20°C. On warming, two new peaks with retention times 3.7 and 3.9 min were formed (the less polar potential prodrug had Rt = 4.1 min). These retention times corresponded with the retention times for prednisolone-21-hemisuccinate and 5-nitro-2-thienylmethanol,

respectively. As in the case of **42**, hydrolysis of an ester was strongly suspected.(**Appendix 1C**).

The nitrothienylmethyl potential prodrugs of the isoquinolin-1-ones **139-141** were too insoluble in methanol for this reductive system to be investigated. Thus a different approach was considered for the release studies on the potential prodrugs.

The success of the NMR-monitored release studies for the indoledione-based compounds was not transferred to the case of the nitrothiophene-based analogues. Tin(II) chloride in deuterated chloroform and methanol at room temperature did not induce the reduction and release of the drug counterpart for any of the nitrothienyl-methyl potential prodrugs.

The method used for the release studies on nitrofuran prodrugs[83] seemed to be a more appropriate system to use. Each nitrothienylmethyl potential prodrug was treated with sodium borohydride in the presence of palladium on carbon in aqueous isopropanol. TLC analysis indicated the disappearance of the potential prodrugs and the presence of the free drugs after 30 min. Proton NMR analysis was carried out for each mixture after reaction. The spectra obtained showed complicated patterns. In each case, the aliphatic region was complicated and few interpretations could be obtained. The peaks corresponding to the nitrothiophene had disappeared in each case.

In these NMR studies, after reduction of **139**, the free isoquinolinone was detected, giving a doublet for 8-H at δ 8.23, followed by the *pseudo* triplet of H-7 at δ 7.62, the doublet of H-5 at δ 7.48, the *pseudo* triplet of H-6 at δ 7.41 and the two doublets of H-3 and H-4 at δ 7.19 and δ 6.59, respectively. These signals were identical to those of isoquinolin-1-one **28** recorded under the same conditions but were distinct from those of the isoquinoline unit in the potential prodrug (**Appendix 19**). Reduction of **140** (**Appendix 20**)and **141** (**Appendix 22**) also gave the free 5-iodo- and 5-bromo-isoquinolin-1-ones **34** (**Appendix 21**), **35** (**Appendix 23**).

^{1}H NMR analysis of the product of reduction of the aspirin potential prodrug **42** (**Appendix 24**) showed two sets of peaks in the aromatic region. It was suspected that one set corresponded to salicylic acid and the other to aspirin, implying that hydrolytic deacetylation took place, either before or after release. The absence of nitrothiophene peaks suggested that the trigger subunit was efficiently reduced, but it is not known whether the drug was released following potential prodrug activation or whether the ester cleavage preceded the trigger reduction (**Appendices 25 and 26**).

Complex results were also obtained after reduction of prednisolone potential prodrug **129** (**Appendix 27**). Aliphatic protons from the steroid could be identified amongst the multiple signals of the aliphatic region. However, the steroid alkene protons, namely H-1, H-2 and H-4, were not found. The reductive conditions could have led to reduction of the two double bonds of the steroid A-ring. The two CH_2 groups of the hemisuccinate linker were masked by another signal rendering impossible to tell whether the two ester bonds present in the molecule were affected by the environment. Therefore no conclusion could be drawn on whether the drug moiety was released after prodrug activation or ester cleavage took place first (**Appendix 28**).

Successful reduction of each of the nitrothiophene-based potential prodrugs was achieved. Release of the drug moiety was observed for in the case of **139**, **140** and **141**, the isoquinoline potential prodrug analogues. However in the case of the two target compounds containing ester bonds **42** and **129**, the order of events could not be determined, *i.e.* there was no evidence for the specific release of the drug moieties after reduction, the reductive ester cleavage before reduction of the trigger was just as probable.

In contrast to the reversible reduction of the indoledione potential prodrugs, the reduction of the nitrothiophenes is essentially irreversible, since aminothiophenes often undergo rapid ring-opening reactions. Thus the effective lifetime of the reduced potential prodrug is much longer, allowing even poor leaving groups to be expelled.

CONCLUSION

Two series of potential prodrugs, designed for bioreductive activation and release of anti-inflammatory and anti-arthritic drugs, have been synthesised for the selective delivery of these drugs to areas of inflammation characterised by low oxygen concentrations.

One series is based on 1,2-dimethyl-5-methoxyindole-4,7-dione unit. The synthesis of the first potential prodrug was achieved by nucleophilic attack from the steroidal drug moiety on the trigger. The introduction of a spacer proved to be necessary due to steric hindrance. The drug counterpart for the four following potential prodrugs were PARP inhibitors, namely isoquinolin-1-one and its 5-substituted analogues. Trigger and effector were linked *via* the Mitsunobu reaction. The site of attachment of the trigger on the drug varied with the analogue used, the ether derivative was obtained for isoquinolin-1-one, N-alkylation took place in the case of 5-bromo and of the Boc-protected 5-aminoisoquinolinones. 5-Iodoisoquinolinone gave both derivatives separately. There is some evidence that the nature of the 5-substituent and the precise reaction conditions have a major effect on the regioselectivity of the reaction.

The second series was based on the 5-nitrothiophene-2-ylmethylene unit. The influence of the nitro group at position 5 influenced the chemistry of the thiophene **119** considerably. Reactions involving **119** as the electrophile failed due to the acidity of the methylene protons and consequently the risk of ring opening. The nucleophilic species derived from **119**, however, reacted with 2-acetoxybenzoyl chloride to give potential prodrug **42**. Coupling of the nitrothienylmethyl succinate active ester **128** with prednisolone generated potential prodrug **129**. Three potential prodrugs were obtained from Mitsunobu reaction between trigger **119** and the isoquinolinone analogues. Each analogue reacted with **119** to give the ether derivatives. No N-alkylation was observed, adding a possible alcohol effect to the speculations made above for the indoledione series.

The release studies were carried out using chemical systems to represent the *in vivo* reductive conditions. The indoledione-isoquinoline ethers released the drug moiety under reductive conditions (tin(II) chloride). The reduction observed proved to be

stoichiometric, no Lewis acid seemed involved. The N-alkylated derivatives and the steroid potential prodrug did not give any signs of release. Although the quinone seemed to be reduced, the half-life of the reduced form was suspected to be short. The release of drugs from **11** is reversible and a leaving group effect was probably at the origin of the lack of reactivity observed in the case of **65**, **39** and **40**.

The nitrothiophene series was reduced with sodium borohydride in the presence of palladium on carbon. Reduction of the nitrothiophene trigger was observed for each target, and release of the isoquinolinone effector moiety was detected for **139**, **140** and **141**. The weakness of potential prodrugs **42** and **129** relied on the presence of ester bonds as linkers. The results obtained by proton NMR analysis together with HPLC data suggested that the ester bond between the trigger and effector unit was hydrolysed under the conditions before reductive trigger activation.

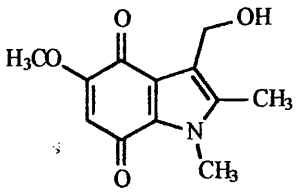
Finally, this study showed that nitrothiophene- and indoledione-based potential prodrugs could be used as potential bioreductively activatable prodrugs. Release of the drug moiety was observed for each series for some of the potential prodrugs. However, testing of the compounds in hypoxic cell culture and *in vivo* is necessary to draw further conclusions on the hypoxia and enzyme selectivity and potential toxicity of the trigger moieties after drug release.

EXPERIMENTAL

MATERIALS AND GENERAL PROCEDURES

Chemicals were purchased from the Aldrich, Fluka, Lancaster and Sigma chemical companies and were used without further purification. All solvents were either GPR or HPLC grade purchased from BDH. Anhydrous solvents were purchased in anhydrous form, or prepared by standard procedures (THF was used freshly distilled from Na/benzophenone). Water refers to singly distilled water. All solvent ratios are given as volume/volume. When anhydrous reagents were used, all glassware was first dried at 120°C, *in vacuo*. ^{1}H and ^{13}C NMR spectra were recorded on JEOL GX270 or VARIAN EX400 spectrometers. Chemical shifts (δ) are reported in parts per million downfield from tetramethylsilane as internal standard. Coupling constants (*J*) are in Hertz and multiplicities are indicated as follows: s (singlet), d (doublet), t (triplet), dd (doublet of doublets), q (quartet), m (multiplet) and br (broad signal). Assignments of ^{13}C NMR spectra were assisted by the use of 135- and 90-DEPT experiments. Thin layer chromatography (TLC) was performed routinely to monitor reaction progress and chemical purity. TLC was performed on pre-coated plates (Merck TLC aluminium sheets 60 F_{254}, Art no. 5554). The spots were visualised using UV light (254 nm or 366 nm). Flash column chromatography was performed according to the method of Still *et al* (1978) using Sorbasil C60-H silica gel purchased from BDH. Columns were packed as a slurry in the eluting solvent and pressure was applied with a Hy Flo pxw-430-T one piston pump. High performance chromatography, for the release studies, was performed using a semi-preparative column Kromasil 10C18, a Jasco PU-986 preparative pump and Jasco UV-975 detector. Methanol was used as the eluant with a flow rate of 5 mL min^{-1}, the injection volume was kept at 20 μL. Melting points (mp) were determined using a Reichert-Jung Thermo Galen Kopfler block and are uncorrected. Infra red spectra were recorded either as a thin liquid film or as a KBr disc using a Perkin Elmer 782 instrument. Ultra Violet spectra were obtained from solutions in chloroform using a Perkin Elmer Lambda 3 spectrometer. Mass spectra were obtained using VG autospec spectrometer. Electron impact ionisation (EI) spectra were recorded typically using an ionising potential of 70 eV. Positive and negative FAB mass spectra were recorded using 3-nitrobenzyl alcohol as the matrix.

In vacuo refers to the use of a water pump, at a pressure typically 15-30 mmHg. For the removal of solvents *in vacuo*, a Büchi rotary evaporator was used and for drying of solids *in vacuo*, a Gallenkamp vacuum oven was used with P_2O_5 as the drying agent, unless otherwise stated. Experiments were conducted at room temperature, unless otherwise stated. Solutions in organic solvents were dried with $MgSO_4$.



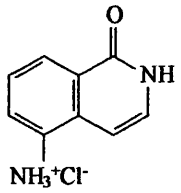
1,2-Dimethyl-3-(hydroxymethyl)-5-methoxyindole-4,7-dione (11)

Method 1: to **54** (3.4 g, 12.3 mmol) in $CHCl_3$ (370 ml) and EtOH (125 ml) was added a solution of sodium dithionite (25.0 g) in water (160 ml). The mixture was stirred vigorously overnight. The organic layer was separated, washed with brine, dried and concentrated to give **55** as a buff solid that was used directly. To a stirred suspension of **55** in toluene (500 ml) under N_2 at –30°C, was added DIBAL-H (1 M solution in toluene; 13.9 g, 97.9 mmol) dropwise during 5 min. The mixture was stirred at this temperature for 2 h. The reaction was quenched by dropwise addition of iron(III) chloride solution (1 M $FeCl_3$/0.1 M HCl, 125 ml) while keeping the temperature below –30°C. The mixture was filtered (Celite®) and the solid was washed with hot CH_2Cl_2. The organic layer was separated, washed with saturated aq. NH_4Cl and dried. Evaporation and chromatography (EtOAc/CH_2Cl_2 4:1) yielded **11** (0.20 g, 6.4%) as an orange-red crystalline solid: Rf 0.3 (EtOAc/CH_2Cl_2 4:1); mp 200-201°C (lit.[51] mp 199-200°C); ^{1}H NMR ($CDCl_3$) δ 5.60 (1 H, s, 6-H), 4.59 (2 H, d, J = 7.0 Hz, CH_2OH), 3.87 (1 H, t, J = 6.7 Hz, OH), 3.88 (3 H, s, OCH_3), 3.81 (3 H, s, NCH_3), 2.21 (3 H, s, 2-CH_3).

Method 2: to $LiAlH_4$ (0.13 g, 3.3 mmol) in THF (20 ml) at 0°C was added **53** (0.22 g, 0.84 mmol) in THF (10 ml). The mixture was allowed to warm to 20°C and was stirred for 30 min. It was then cooled to 0°C and the reaction was quenched by addition of water (0.1 ml), 1 M NaOH (0.1 ml) and silica gel (1.0 g). The granular precipitate was filtered off (Celite®). The filtrate was dried and evaporation yielded **56** (0.15 g, 80%) as an off-white powder which was used directly in the next step without further purification: ^{1}H NMR ($CDCl_3$) δ 6.86 (1 H, d, J = 8.7 Hz, 6-H), 6.59 (1 H, d, J = 8.7 Hz, 7-H), 4.78 (2 H, s, CH_2), 3.88 (3 H, s, OCH_3), 3.80 (3 H, s, NCH_3), 2.25 (3 H, s, 2-CH_3).

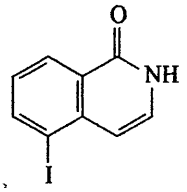
To **56** (0.2 g, 0.91 mmol) in acetone (40 ml) was added potassium nitrosodisulfonate (0.9 g, 3.6 mmol) in phosphate buffer (0.3 M, pH 6.7, 40 ml). The mixture was stirred for 1h. The acetone was evaporated *in vacuo*. The resulting residue was extracted with CH_2Cl_2 and washed with water. The organic layer was dried. Evaporation, chromatography (EtOAc/CH_2Cl_2 4:1) and recrystallisation (CH_2Cl_2/hexane) yielded **11** (0.16 g, 75%) as an orange-red solid (characterisation above).

Method 3: to a suspension of **62** (0.10 g, 0.43 mmol) in MeOH (40 ml, degassed) was added $NaBH_4$ (0.16 g, 4.3 mmol) under dry Ar. The solution was stirred for 1 h and aerated prior to the addition of water (20 ml). The mixture was extracted with CH_2Cl_2 (3 x 50 ml) and dried. Evaporation, chromatography (EtOAc) and recrystallisation (EtOAc) afforded **11** (0.05 g, 33%) as an orange-red solid (characterisation above).



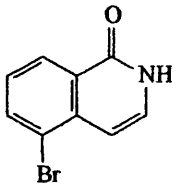
5-Aminoisoquinolin-1-one hydrochloride (33')

Compound **86** (0.15 g, 0.8 mmol) in EtOH (12 ml) and conc. HCl (0.4 ml) was stirred under H_2 in the presence of palladium on charcoal (0.1 g) for 1 h. The suspension was filtered through Celite®. The Celite® pad and residue were heated in water (200 ml). The resulting hot suspension was filtered through a second Celite® pad. Evaporation of the solvent from the combined filtrates gave **33'** (0.12 g, 95%) as off-white crystals: mp >230°C (lit.[111] mp >230°C); ^{1}H NMR (D_2O) δ 8.29 (1 H, d, J = 8.0 Hz, 8-H), 7.77 (1 H, d, J = 8.0 Hz, 6-H), 7.63 (1 H, t, J = 8.0 Hz, 7-H), 7.42 (1 H, d, J = 7.5 Hz, 3-H), 6.80 (1 H, d, J = 7.5 Hz, 4-H); MS (EI) *m/z* 160.0641 (M), ($C_9H_8N_2O$ requires 160.0636).



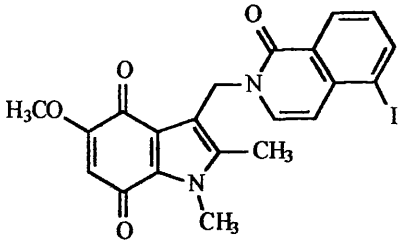
5-Iodoisoquinolin-1-one (34)

Compound **82** (0.3 g, 1.1 mmol) was added to a solution of 2-methoxyethanol (50 ml) saturated with ammonia. The solution was boiled under reflux for 24 h, with periodic cooling and re-saturation with ammonia. Evaporation yielded **34** as beige crystals (0.2 g, 68%): mp 239-242°C (lit.[83] mp 238-244°C); ^{1}H NMR ($CDCl_3$) δ 11.20 (1 H, s, NH), 8.35 (1 H, dd, J = 7.5, 1.1 Hz, 8-H), 8.15 (1 H, dd, J = 7.5, 1.1 Hz, 6-H), 7.17 (1 H, t, J = 7.5 Hz, 7-H), 7.13 (1 H, d, J = 7.4 Hz, 3-H), 6.67 (1 H, d, J = 7.4 Hz, 4-H).



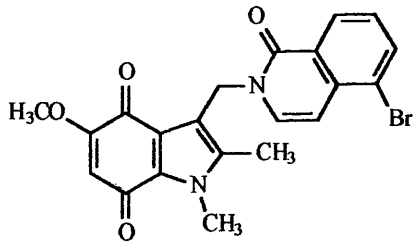
5-Bromoisoquinolin-1-one (35)

Compound **83** (0.3 g, 1.1 mmol) was added to a solution of 2-methoxyethanol (50 ml) saturated with ammonia. The solution was boiled under reflux for 24 h, with periodic cooling and re-saturation with ammonia. Evaporation yielded **35** (0.2 g, 71%) as beige crystals: mp 240-243°C (lit.[113] mp 242-244°C); ^{1}H NMR ($CDCl_3$) δ 11.17 (1 H, s, NH), 8.21 (1 H, d, J = 8.0 Hz, 8-H), 8.05 (1 H, d, J = 8.0 Hz, 6-H), 7.41 (1 H, d, J = 7.5 Hz, 3-H), 7.35 (1 H, t, J = 8.0 Hz, 7-H), 6.67 (1 H, d, J = 7.5 Hz, 4-H).



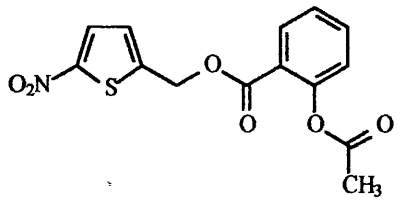
1,2-Dimethyl-3-(5-iodo-1-oxoisoquinolin-2-ylmethyl)-5-methoxyindole-4,7-dione (39).

Diethyl azodicarboxylate (0.12 ml, 0.7 mmol) was added dropwise to 5-iodoisoquinolin-1-one **34** (0.1 g, 0.4 mmol) and PPh_3 (0.19 g, 0.7 mmol) in dry THF (20 ml) under dry Ar. The mixture was stirred for 15 min and **11** (0.085 g, 0.4 mmol) was added. The mixture was stirred overnight. Evaporation and chromatography (EtOAc) afforded **39** (63 mg, 36%) as a purple powder: Rf 0.46 (EtOAc); mp >230°C; IR 3096, 2923, 1702, 1470, 1380 cm^{-1}; ^1H NMR ($CDCl_3$) δ. 8.38 (1 H, d, J = 8.2 Hz, 8-H), 8.11 (H, dd, J = 7.9, 1.1 Hz, isoquinoline 6-H), 7.80 (1 H, d, J = 7.9 Hz, isoquinoline 3-H), 7.12 (1 H, t, J = 7.8 Hz, isoquinoline 7-H), 6.66 (1 H, d, J = 7.9 Hz, isoquinoline 4-H), 5.62 (1 H, s, indole 6-H), 5.29 (2 H, s, CH_2), 3.88 (3 H, s, CH_3O), 3.81 (3 H, s, CH_3N), 2.47 (3 H, s, CH_3); ^{13}C NMR ($CDCl_3$) δ 178.1 (indole 7-CO), 161.1 (indole 4-CO), 159.0 (isoquinoline 1-CO), 142.5 (indole 5-C), 138.9 (isoquinoline 8a-C), 138.7 (isoquinoline 3-CH), 134.1 (isoquinoline 6-CH), 128.7 (isoquinoline 4a-C), 128.0 (indole 7a-C), 127.2 (isoquinoline 7-CH and indole 2-C), 126.7 (isoquinoline 8-CH), 121.1 (isoquinoline 5-C and indole 3a-C), 109.1 (isoquinoline 4-CH and indole 3-C), 106.5 (indole 6-CH), 56.5 (CH_3O), 42.2 (CH_3N), 32.6 (CH_2), 10.2 (CH_3); MS (FAB +ve) *m/z* 489.0309 (M+H), ($C_{21}H_{18}N_2O_4I$ requires 489.0311).



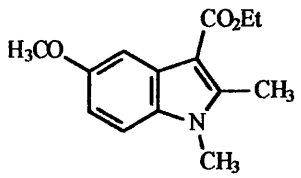
1,2-Dimethyl-3-(5-bromo-1-oxoisoquinolin-2-ylmethyl)-5-methoxyindole-4,7-dione (40)

Diethyl azodicarboxylate (0.07 ml, 0.4 mmol) was added dropwise to 5-bromoisoquinolin-1-one **35** (0.05 g, 0.2 mmol) and PPh_3 (0.11 g, 0.4 mmol) in dry THF (20 ml) under dry Ar. The mixture was stirred for 15 min and **11** (0.05 g, 0.2 mmol) was added. The mixture was stirred overnight. Evaporation and chromatography (EtOAc) afforded **40** (38 mg, 40%) as a purple powder: Rf 0.35 (EtOAc/hexane 10:1); mp 278-280°C; IR 3070, 2999, 1695, 1693, 1676, 1465, 1399 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.36 (1 H, d, J = 7.8 Hz, isoquinoline 8-H), 7.84 (1 H, dd, J = 7.3, 0.9 Hz, isoquinoline 6-H), 7.82 (1 H, d, J = 8.0 Hz, isoquinoline 3-H), 7.27 (1 H, t, J = 8.2 Hz, isoquinoline 7-H), 6.77 (1 H, d, J = 8.0 Hz, isoquinoline 4-H), 5.62 (1 H, s, indole 6-H), 5.30 (2 H, s, CH_2), 3.88 (3 H, s, CH_3O), 3.81 (3 H, s, CH_3N), 2.47 (3 H, s, indole 2-CH_3); ^{13}C NMR ($CDCl_3$) δ 178.2 (indole 7-CO), 161.2 (indole 4-CO), 159.3 (isoquinoline 1-CO), 138.8 (isoquinoline 8a-C), 136.3 (indole 5-C), 135.6 (isoquinoline 3-CH), 134.1 (isoquinoline 6-CH), 128.8 (isoquinoline 4a-C), 128.5 (indole 2-C), 128.1 (indole 7a-C), 127.4 (isoquinoline 7-CH), 126.8 (isoquinoline 8-CH), 121.2 (isoquinoline 5-C), 120.4 (indole 3a-C), 116.1 (indole 3-C), 106.6 (isoquinoline 4-CH), 104.3 (indole 6-CH), 56.5 (CH_3O), 42.2 (CH_3N), 32.6 (CH_2), 10.2 (CH_3); MS (FAB +ve) *m/z* 441.0443 (M+H), ($C_{21}H_{18}N_2O_4Br$ requires 441.0449).



5-Nitrothien-2-ylmethyl 2-acetoxybenzoate (42)

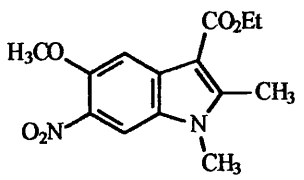
Triethylamine was added (0.65 ml, 4.7 mmol) to **119** (0.5 g, 301 mmol) in CH_2Cl_2 (5 ml) at 0°C. The mixture was stirred for 10 min at 0°C. 2-Acetoxy benzoyl chloride (0.62 g, 3.1 mmol) in CH_2Cl_2 (5 ml), was added. The resulting mixture was stirred at 20°C for 1.5 h, and the solvent was evaporated. The residue in CH_2Cl_2 was washed with HCl 3 M, aqueous $NaHCO_3$, and brine. It was then dried and the solvent was evaporated. Chromatography (hexane/EtOAc 1:1) yielded **42** (0.36 g, 35%) as a yellow oil: Rf 0.5 (hexane / EtOAc 1:1); IR 3100, 2960, 1715, 1750, 1600, 1500, 1450, 1370, 740, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.04 (1H, dd, J = 1.5, 7.8 Hz, Ph 6-H), 7.83 (1 H, d, J = 3.9 Hz, thiophene 4-H), 7.61 (1H, ddd, J = 1.5, 7.4, 8.2 Hz, Ph 4-H), 7.33 (1H, ddd, J = 1.1, 7.4, 7.8 Hz, Ph 5-H), 7.12 (1 H, dd, J = 1.1, 8.2 Hz, 3-H), 7.07 (1 H, d, J = 3.9 Hz, thiophene 3-H), 5.41 (2 H, s, CH_2), 2.31 (3 H, s, CH_3); ^{13}C NMR ($CDCl_3$) δ 169.3 (CO), 163.4 (CO), 150.8 (thiophene 5-C), 145.4 (Ph 2-C), 134.4 (thiophene 2-C), 131.6 (Ph 4-CH and thiophene 4-CH), 128.0 (Ph 6-CH), 126.7 (thiophene 3-CH), 126.0 (Ph 1-C), 123.8 (Ph 5-CH), 122.0 (Ph 3-CH), 60.8 (CH_2), 21.0 (CH_3); MS (FAB +ve) *m/z* 322.0385 (M+H) ($C_{14}H_{12}NO_6S$ requires 322.0385). Anal. C 52.28%, H 3.42%, N 4.35% ($C_{14}H_{11}N\ O_6S$ requires C 52.33%, H 3.42%, N 4.36%).

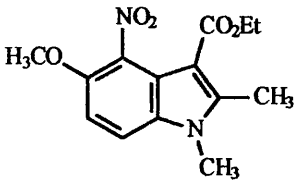


Ethyl 1,2-dimethyl-5-methoxyindole-3-carboxylate (49)

Ethyl 5-hydroxy-2-methylindole-3-carboxylate (**48**) (6.0 g, 27.5 mmol) in dry DMF (50 ml) was added under N_2 to a stirred suspension of KH (3.3 g, 82.5 mmol) in dry DMF (200 ml) at 0°C. The mixture was stirred for 45 min. Iodomethane (11.7 g, 82.4

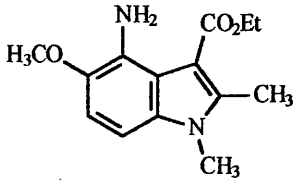
mmol) was added dropwise at 0°C and the mixture was allowed to warm to 20°C during 4 h. Saturated aqueous NH_4Cl was added and the mixture was extracted with EtOAc (3×75 ml). The EtOAc solution was washed twice with water and dried. Evaporation and chromatography (EtOAc) gave **49** as a white solid (5.4 g, 80 %): Rf 0.5 (EtOAc); mp 119-120°C (lit.[51] mp 119-121°C); ^{1}H NMR ($CDCl_3$) δ 7.66 (1 H, d, J = 2.5 Hz, 4-H), 7.17 (1 H, d, J = 8.8 Hz, 7-H), 6.87 (1 H, dd, J = 8.8, 2.5 Hz, 6-H), 4.39 (2 H, q, J = 7.1 Hz, CH_2), 3.88 (3 H, s, OCH_3), 3.66 (3 H, s, NCH_3), 2.74 (3 H, s, 2-CH_3), 1.45 (3 H, t, J = 7.1 Hz CH_2CH_3).





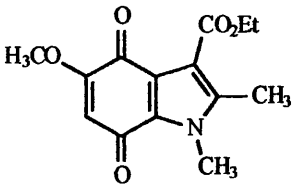
Ethyl 1,2-dimethyl-5-methoxy-6-nitroindole-3-carboxylate (51) and ethyl 1,2-dimethyl-5-methoxy-4-nitroindole-3-carboxylate (52).

To **51** (5.1 g, 20.6 mmol) in AcOH (80 ml), cooled to –10°C, was added a mixture of conc. HNO_3 (11 ml) and AcOH (41 ml). The mixture was stirred for 2 h, while warming to 20°C. The yellow suspension was poured onto an ice/water mixture and, after 15 min, the solid was collected by filtration, and dried. Chromatography (EtOAc/light petroleum 1:1) yielded **51** (0.8 g, 14%): Rf 0.6 (EtOAc); mp 138-139°C (lit.[45] mp 139-141°C); ^{1}H NMR ($CDCl_3$) δ 7.96 (1 H, s, 7-H), 7.79 (1 H, s, 4-H), 4.40 (2 H, q, J = 7.0 Hz, CH_2), 4.02 (3 H, s, OCH_3), 3.73 (3 H, s, NCH_3), 2.79 (3 H, s, 2-CH_3), 1.46 (3 H, t, J = 7.0 Hz CH_2CH_3). Further elution gave **52** (3.8 g, 63%): Rf 0.4 (EtOAc); mp 187-188°C (lit.[51] mp 189-192°C); ^{1}H NMR ($CDCl_3$) δ 7.30 (1 H, d, J = 9.1 Hz, 7-H), 6.93 (1 H, d, J = 9.1 Hz, 6-H), 4.29 (2 H, q, J = 7.1 Hz, CH_2), 3.91 (3 H, s, OCH_3), 3.66 (3 H, s, NCH_3), 2.67 (3 H, s, 2-CH_3), 1.36 (3 H, t, J = 7.1 Hz CH_2CH_3).



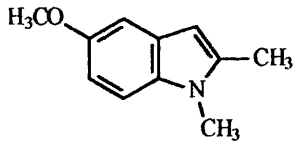
Ethyl 4-amino-1,2–dimethyl-5-methoxyindole-3-carboxylate (53)

To **52** (2.18 g, 7.5 mmol) in EtOH (190 ml) were added HCl (conc., 10ml) and tin powder (4.0 g, 33.6 mmol). The mixture was heated under reflux for 30 min. Upon cooling, the solution was decanted from the excess of tin and was neutralised with aqueous $NaHCO_3$. The suspension was added to an equal volume of water, was stirred overnight with CH_2Cl_2 (200 ml) and filtered (Celite). The organic layer was dried ($MgSO_4$) and concentrated. Evaporation and chromatography (EtOAc) yielded **53** as a light yellow crystalline solid (1.56 g, 80%): Rf 0.6 (EtOAc); mp 96-97°C (lit.[51] mp 96-98°C); ^{1}H NMR ($CDCl_3$) δ 6.88 (1 H, d, J = 8.7 Hz, 7-H), 6.52 (1 H, d, J = 8.7 Hz, 6-H), 5.68 (2 H, br s, NH_2), 4.36 (2 H, q, J = 7.0 Hz, CH_2), 3.87 (3 H, s, OCH_3), 3.58 (3 H, s, NCH_3), 2.64 (3 H, s, 2-CH_3), 1.41 (3 H, t, J = 7.0 Hz, CH_2CH_3).



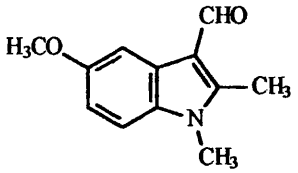
Ethyl 1,2–dimethyl–4,7–dioxo-5-methoxyindole-3-carboxylate (54)

To **53** (3.2 g, 12.2 mmol) in Me_2CO (400 ml) was added potassium nitrosodisulfonate (5 g, excess) in phosphate buffer (NaH_2PO_4/Na_2HPO_4) (0.3 M, 400 ml, pH 6.0). The mixture was stirred for 1 h. The excess Me_2CO was removed *in vacuo*. The residue was extracted with CH_2Cl_2 (3×75 ml) which was washed with water and dried. Evaporation and recrystallisation (CH_2Cl_2/light petroleum, 2:3) gave **54** (3.3 g, 97%) as an orange-yellow crystalline solid: Rf 0.4 (EtOAc); mp 202-203°C (lit.[51] mp 202-204°C); ^{1}H NMR ($CDCl_3$) δ 5.64 (1 H, s, 6-H), 4.36 (2 H, q, J = 7.1 Hz, CH_2), 3.91 (3 H, s, OCH_3), 3.81 (3 H, s, NCH_3), 2.44 (3 H, s, 2-CH_3), 1.40 (3 H, t, J = 7.1 Hz, CH_2CH_3).



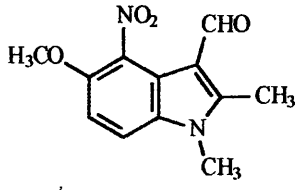
1,2-Dimethyl-5-methoxyindole (58)

5-Methoxy-2-methylindole **57** (0.1 g, 0.62 mmol) was added slowly under dry Ar to a stirred suspension of NaH (0.027 g of a 60% dispersion, 0.68 mmol) in DMF (15 ml). The suspension was heated at 45°C for 10 min and cooled to 20°C. MeI (0.33 ml, 5.3 mmol) was added during 5 min. The solution was then heated at 60°C for 1 h, cooled, poured onto cold (0°C) $NaHSO_4$ (aqueous, 10%, 5 ml), extracted with EtOAc (3x15 ml), dried and evaporated. Chromatography (EtOAc/hexane 3:97) gave **58** (0.09 g, 80%) as a pale buff solid: Rf 0.5 (EtOAc/hexane 3:97); mp 70-73°C (lit.[50] mp 73-74°C); ^{1}H NMR ($CDCl_3$) δ 7.06 (1 H, d, J = 8.8 Hz, 7-H), 6.93 (1 H, d, J = 2.4 Hz, 4-H), 6.73 (1 H, dd, J = 2.4, 8.8 Hz, 6-H), 6.16 (1 H, s, 3-H), 3.82 (3 H, s, OCH_3), 3.59 (3 H, s, NCH_3), 2.37 (3 H, s, 2-CH_3).



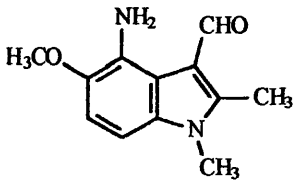
1,2-Dimethyl-5-methoxyindole-3-carboxaldehyde (59)

Anhydrous DMF (0.088 ml, 1.1 mmol) was added to **58** (0.1 g, 0.57 mmol) in CH_2Cl_2 (3 ml), was added and the mixture was stirred for 5 min. $POCl_3$ (0.1 ml, 1.1 mmol) was then added dropwise. The solution was heated under reflux for 2 h and cooled, aqueous NaOAc (1.0 M, 10 ml) was added and the solution was stirred for 2.5 h. The solution was extracted with EtOAc and dried. Evaporation and chromatography (EtOAc/hexane 1:1) gave **59** (0.05 g, 43%) as an off-white solid: Rf 0.5 (EtOAc); mp 115-117°C (lit.[50] mp 108-110°C); ^{1}H NMR ($CDCl_3$) δ 10.1 (1 H, s, CHO), 7.80 (1 H, d, J = 2.3 Hz, 4-H), 7.17 (1 H, d, J = 8.9 Hz, 7-H), 6.89 (1 H, dd, J = 2.3, 8.9 Hz, 6-H), 3.89 (3 H, s, OCH_3), 3.63 (3 H, s, NCH_3), 2.62 (3 H, s, 2-CH_3).



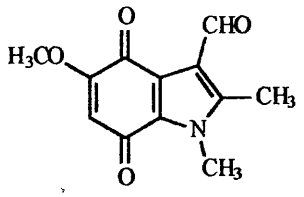
1,2-Dimethyl-5-methoxy-4-nitroindole-3-carboxaldehyde (60)

Compound **59** (0.70 g, 3.4 mmol) in acetic acid (55 ml) was cooled to 5°C and yellow fuming nitric acid (2.0 ml) in AcOH (8.2 ml) was added dropwise during 5 min. The temperature was allowed to rise to 20°C during 3 h, the mixture was poured onto crushed ice and the precipitate was collected by filtration. Chromatography (EtOAc/hexane 2:1) gave **60** (0.43 g, 60%) as a pale yellow solid: Rf 0.42 (EtOAc); mp 235-237°C (lit.[50] mp 236-238°C); ^{1}H NMR (DMSO) δ 9.90 (1 H, s, CHO), 7.75 (1 H, d, J = 9.0 Hz, 7-H), 7.25 (1 H, d, J = 9.0 Hz, 6-H), 3.89 (3 H, s, OCH_3), 3.76 (3 H, s, NCH_3), 2.71 (3 H, s, 2-CH_3).



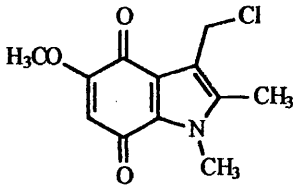
4-Amino-1,2-dimethyl-5-methoxyindole-3-carboxaldehyde (61)

Powdered tin (0.35 g, 2.9 mmol) was added to **60** (0.15 g, 0.60 mmol) in EtOH (15 ml), followed by aqueous HCl (3.0 M, 5 ml), and the solution was heated under reflux for 1 h. Water (45 ml) was added and the solution neutralised with aqueous $NaHCO_3$, extracted with $CHCl_3$ (3 x 100 ml), dried and evaporated. Chromatography (EtOAc/Hexane 1:1) gave **61** (0.10 g, 62%) as a pale yellow solid: Rf 0.6 (EtOAc); mp 155-157°C (lit.[50] mp 152-153°C); ^{1}H NMR (DMSO) δ 9.71 (1 H, s, CHO), 6.85 (1 H, d, J = 9.0. Hz, 6-H), 6.56 (1 H, d, J = 9.0 Hz, 7-H), 6.0 (2 H, s, NH_2), 3.75 (3 H, s, OCH_3), 3.59 (3 H, s, NCH_3), 2.61 (3 H, s, 2-CH_3).



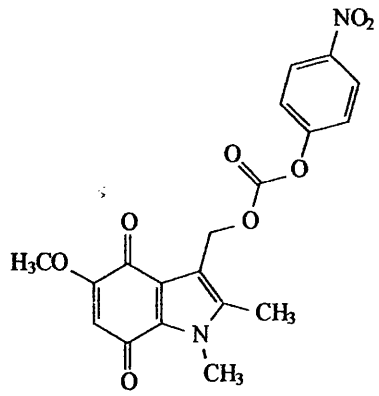
1,2-Dimethyl-3-formyl-5-methoxyindole-4,7-dione (62)

To compound **61** (0.15 g, 0.68 mmol) in acetone (30.5 ml) was added potassium nitrosodisulfonate (0.92 g, 3.4 mmol) in NaH_2PO_4/Na_2HPO_4 buffer (30.5 ml, 0.3 M, pH 6) and the solution was stirred for 1 h. The acetone was evaporated at 30°C. The precipitate was collected by filtration and was washed with H_2O and cold MeOH to give **62** (0.12 g, 75%) as an orange solid: Rf 0.5 (EtOAc); mp 240-242°C (lit.[50] mp 239-242°C); ^{1}H NMR (DMSO) δ 10.37 (1 H, s, CHO), 5.89 (1 H, s, 6-H), 3.88 (3 H, s, OCH_3), 3.82 (3 H, s, NCH_3), 2.50 (3 H, s, 2-CH_3).



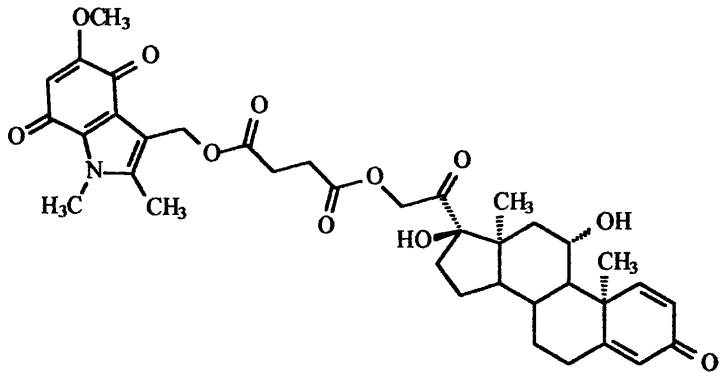
3-(Chloromethyl)-1,2-dimethyl-5-methoxyindole-4,7-dione (63)

Compound **11** (0.5 g, 2 mmol) was stirred with $SOCl_2$ (5 ml) for 30 min. The solution was then evaporated *in vacuo*, dissolved in EtOAc (25 ml), and evaporated to dryness. This procedure was repeated twice. Recrystallisation (EtOAc) gave **63** (0.42 g, 79 %) as an orange solid: Rf 0.25 (EtOAc); mp 203-204°C (lit.[51] mp 204-205°C); ^{1}H NMR ($CDCl_3$) δ 5.62 (1 H, s, 6-H), 4.86 (2 H, s, CH_2), 3.89 (3 H, s, OCH_3), 3.80 (3 H, s, NCH_3), 2.28 (3 H, s, 2-CH_3).



(1,2-Dimethyl-4,7-dioxo-5-methoxyindol-3-yl)methyl 4-nitrophenyl carbonate (64)

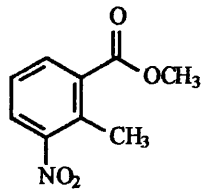
4-Nitrophenyl chloroformate (0.085 g, 0.42 mmol) in THF (10 ml) was added slowly to **11** (0.1 g, 0.42 mmol) and pyridine (0.033 ml, 042 mmol) in THF (15 ml) under Ar. The mixture was stirred for 2 d. The evaporation residue in EtOAc was washed with water and brine and dried. Evaporation and chromatography (EtOAc) yielded **64** (0.13 g, 78%) as a yellow oil: Rf 0.52 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 8.15 (2 H, d, J = 9.1 Hz, Ph 3'/5'-H_2), 6.94 (2 H, d, J = 9.1 Hz, Ph 2'/6'-H_2), 5.66 (1 H, s, indole 6-H), 5.46 (2 H, s, CH_2), 3.93 (3 H, s, CH_3O), 3.82 (3 H, s, NCH_3), 2.34 (3 H, s, indole 2-CH_3).



(1,2-Dimethyl-4,7-dioxo-5-methoxyindol-3-yl)methyl prednisolon-21-yl butanedioate or 4-(1,2-dimethyl-4,7-dioxo-5-methoxyindol-3-ylmethoxy)-21-O-(4-oxobutanoyl)prednisolone (65)

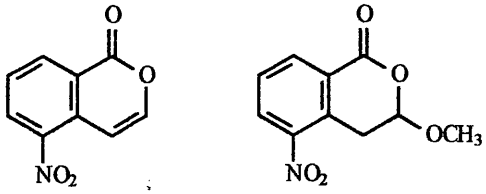
Compound **63** (0.20 g, 0.79 mmol) was dissolved in DMF (50 ml). Prednisolone-21-hemisuccinate sodium salt (0.38 g, 0.79 mmol) was added. The mixture was heated under reflux for 24 h. The solution was allowed to cool and the solvent was

evaporated. Chromatography (EtOAc/CH_2Cl_2 4:1) gave **65** as a bright orange glassy material (0.21 g, 40%): IR (KBr) 3450, 2920, 1730, 1650, 1490, 1220, 1150, 1110, 1050 cm^{-1}; ^1H NMR (DMSO) δ 7.32 (1 H, d, J = 10.5 Hz, steroid 2-H), 6.17 (1 H, d, J = 10.6 Hz, steroid 1-H), 5.92 (1 H, s, steroid 4-H), 5.77 (1 H, s, indole 6-H), 5.16 (2 H, s, CH_2), 5.04 (1 H, d, J = 17.5 Hz) and 4.71 (1 H, d, J = 17.5 Hz) (steroid 21-H_2), 4.27 (1 H, s, steroid 11-H), 3.98 (3 H, s, OCH_3), 3.85 (3 H, s, NCH_3), 3.65 (2 H, m, steroid 6-H_2), 2.8 (4 H, m, butanedioate CH_2CH_2), 2.4 (2 H, m, steroid 16-H_2), 2.24 (3 H, s, indole 2-CH_3), 2.15 (2 H, m, steroid 15-H_2), 2.05 (1 H, m, steroid 9-H), 1.75 (2 H, m, steroid 12-H_2), 1.45 (2 H, m, steroid 7-H_2), 1.38 (3 H, s, steroid 19-CH_3), 1.13 (1 H, m, steroid 8-H), 1.05 (1 H, m, steroid 14-H), 0.76 (3 H, s, steroid 18-CH_3); ^{13}C NMR ($CDCl_3$) δ 204.9 (steroid 20-CO), 186.1 (steroid 3-CO), 178.0 (indole 7-CO), 172 (ester CO), 170.9 (ester CO), 159.7 (indole 4-C), 157.1 (steroid 1-CH), 138.2 (indole 5-C), 129.3 (indole 2-C), 127.7 (indole 3a-C), 122.3 (steroid 2-CH), 121.8 (steroid 4-CH), 115.7 (indole 3-C), 106.8 (indole 6-CH), 89.92 (steroid 17-C), 70.3 (steroid 11-CH), 68.5 (steroid 21-CH_2), 57.3 (CH_3O), 56.7 (steroid 9-CH), 55.8 (steroid 5-CH_2), 51.7 (steroid 14-CH), 48.0 (steroid 13-C), 44.6 (steroid 10-C), 39.8 (steroid 12-CH_2), 34.6 (steroid 16-CH_2), 34.0 (steroid 8-CH), 32.5 (NCH_3 and steroid 7-CH_2), 31 (steroid 6-CH_2), 29.4 (butanedioate 2-CH_2 and 3-CH_2), 24.3 (steroid 15-CH_2), 21.4 (steroid 19-CH_3), 17.7 (steroid 18-CH_3), 9.9 (indole 2-CH_3); MS (FAB +ve) *m/z* 678 (M+H).



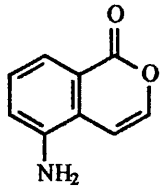
Methyl 2-methyl-3-nitrobenzoate (76)

$SOCl_2$ (6.84 ml, 0.9 mmol) was added dropwise to 2-methyl-3-nitrobenzoic acid **75** (10.0 g, 0.55 mmol) in MeOH (200 ml) and the mixture was boiled under reflux overnight. Evaporation gave **76** as a beige solid (9.0 g, 83%): mp 63-65°C (lit.[114] mp 64-65°C); ^1H NMR ($CDCl_3$) δ.8.00 (1 H, d, J = 7.8 Hz, 4-H), 7.85 (1 H, d, J = 7.8 Hz, 6-H), 7.39 (1 H, t, J = 7.8 Hz, 5-H), 3.94 (3 H, s, OCH_3), 2.61 (3 H, s, 2-CH_3).



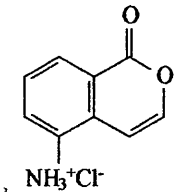
5-Nitroisocoumarin (78) and 3-methoxy-5-nitro-3,4-dihydroisocoumarin (79)

Dimethylformamide dimethyl acetal (21.5 ml, 160 mmol) was added to **76** (9.0 g, 46 mmol) in DMF (30 ml) and the mixture was boiled under refluxed for 20 h. Evaporation and chromatography (hexane/EtOAc 10:1) gave **78** (2.82 g, 32%) as yellow crystals: mp 171-172°C (lit.[108] mp 173-174°C); ^{1}H NMR ($CDCl_3$) δ 8.64 (1 H, ddd, J = 7.8, 1.5, 0.8 Hz, 8-H), 8.51 (1 H, dd, J = 8.2, 1.5 Hz, 6-H), 7.68 (1 H, t, J = 8.2 Hz, 7-H), 7.44 (1 H, d, J = 5.8 Hz, 3-H), 7.39 (1 H, d, J = 5.8, 0.8 Hz, 4-H). Further elution gave **79** (0.90 g, 10%) as yellow crystals: mp 111-112°C (lit.[110] mp 111.5-112.5°C); ^{1}H NMR ($CDCl_3$) δ 8.48 (1 H, d, J = 8.0 Hz, 6-H), 8.28 (1 H, d, J = 8.0 Hz, 8-H), 7.59 (1 H, t, J = 8.0 Hz, 7-H), 5.52 (1 H, t, J = 3.1 Hz, 3-H), 3.59 (2 H, d, J = 3.1 Hz, 4-H_2), 3.55 (3 H, s, CH_3).



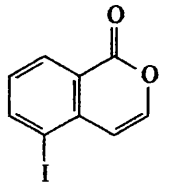
5-Aminoisocoumarin (80)

2 M aqueous HCl (4 ml) was added to **78** (1.67 g, 8.7 mmol) in dry THF (40 ml). Palladium on charcoal (0.25 g) was added and the flask was exposed to hydrogen for 1 h after which the solution was filtered (Celite®). The evaporation residue in CH_2Cl_2 was washed with aq. $NaHCO_3$. The aqueous layer was re-extracted with CH_2Cl_2. The combined organic phases were dried. Evaporation yielded **80** (1.12 g, 80%) as pale yellow crystals: mp 191-192°C (lit.[114] mp 194-195°C); ^{1}H NMR ($CDCl_3$) δ 7.76 (1 H, d, J = 8.1 Hz, 8-H), 7.32 (1 H, t, J = 8.1 Hz, 7-H), 7.25 (1 H, d, J = 6.1 Hz, 3-H), 7.04 (1 H, d, J = 8.1 Hz, 6-H), 6.43 (1 H, d, J = 6.1 Hz, 4-H), 3.96 (2 H, s, NH_2).



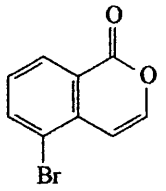
5-Aminoisocoumarin hydrochloride (80')

HCl was passed through **80** (0.15 g, 0.9 mmol) in $CHCl_3$ (50 ml) for 1 h. The solid formed was filtered off to give **80'** (0.17 g, 95%) as white crystals: mp 161-164°C; ^1H NMR ($CDCl_3$) δ 8.14 (1 H, d, J = 8.2. Hz, 8-H), 7.88 (1 H, t, J = 8.2. Hz, 7-H), 7.53 (1 H, t, J = 7.7 Hz, 6-H), 7.39 (1 H,d, J = 6.7 Hz, 3-H), 6.98 (1 H, d, J = 6.7 Hz, 4-H), 2.59 (3 H, s, NH_3); MS (EI) *m/z* 161.0479 (M), ($C_9H_8O_2N$ requires 161.0476).



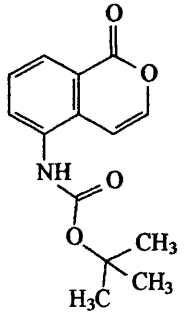
5-Iodoisocoumarin (82)

A solution of $NaNO_2$ (2.6 g, 37.3 mmol) in water (200 ml) was added to a solution of **80** (7.0 g, 43.4 mmol) in HCl (50 % solution, 250 ml) at 0°C, maintaining the temperature of the solution below 5°C. A chilled solution of KI (10.0 g, 60 mmol) and CuI in water (250 ml) was added slowly. The solution was stirred for 2 h before extraction with EtOAc. Evaporation and chromatography (hexane/EtOAc 4:1) yielded **82** (8.3 g, 70%) as off-white crystals: mp 155-156 °C (lit.[83] mp 160-161°C); ^1H NMR (DMSO) δ 8.31 (1 H, d, J = 7.9 Hz, 8-H), 8.22 (1 H, d, J = 7.6 Hz, 6-H), 7.75 (1 H, d, J = 5.9 Hz, 4-H), 7.37 (1 H, d, J = 7.9 Hz, 7-H), 6.75 (1 H, d, J = 5.9 Hz, 3-H).



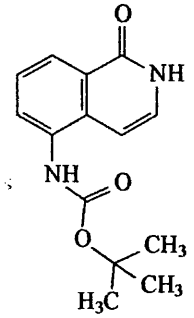
5-Bromoisocoumarin (83)

Compound **80** (2.5 g, 15.4 mmol) was dissolved in 2 M aq. H_2SO_4 (80 ml). The solution was cooled to 0°C. A solution of $NaNO_2$ (1.05 g, 15.4 mmol) in water (5 ml) was added maintaining the temperature below 5°C. KBr (3.6 g, 31 mmol) and CuBr (4.4 g, 30.9 mmol) were added slowly to the solution. The reaction mixture was stirred for 2 h and extracted with EtOAc. Evaporation and chromatography (hexane/EtOAc 6:1) gave **83** (1.4 g, 37%) as off white crystals: mp 135-137°C (lit.[113] 113-115°C); ^{1}H NMR ($CDCl_3$) δ 8.31 (1 H, d, J = 8.1 Hz, 8-H), 7.95 (1 H, d, J = 8.1 Hz, 6-H), 7.41 (1 H, t, J = 8.1 Hz, 7-H), 7.36 (1 H, d, J = 6.2 Hz, 3-H), 6.85 (1H, d, J = 6.2 Hz, 4-H).



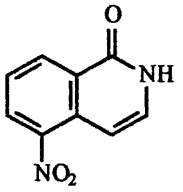
5-(1,1-Dimethylethoxycarbonylamino)isocoumarin (84)

To **80** (0.11 g, 0.8 mmol) in CH_2Cl_2 (20 ml) was added DMF (1.5 ml) and Et_3N (50 µl). The solution was cooled to 0°C and di-*tert*-butyl dicarbonate (0.30 g, 1.4 mmol) in CH_2Cl_2 (1 ml) was added in small quantities over 4 d. The evaporation residue in EtOAc was washed with water and brine and dried. Evaporation and chromatography (EtOAc/hexane 1:4) gave **84** (0.12 g, 60%) as a yellow powder: mp 190-192°C (lit.[113] mp 188-190°C); ^{1}H NMR ($CDCl_3$) δ 8.10 (1 H, d, J = 8.0 Hz, 8-H), 8.05 (1 H, d, J = 6.9 Hz, 6-H), 7.50 (1 H, t, J = 8.0 Hz, 7-H), 7.30 (1 H, d, J = 6.0 Hz, 3-H), 6.53 (1 H, d, J = 6.0 Hz, 4-H), 6.47 (1 H, s, NH), 1.53 (9 H, s, Bu^t).



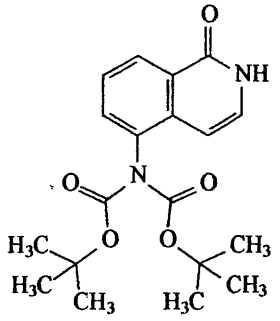
5-(1,1-Dimethylethoxycarbonylamino)isoquinolin-1-one (85)

Et_3N (1.56 ml, 0.1 mmol) was added to **33'** (0.6 g, 3.7 mmol) in DMF (40 ml) and the solution was cooled to 0°C. Di-*tert*-butyl dicarbonate (2.45 g, 0.1mmol) was added in small quantities over 2 d. The evaporation residue in EtOAc was washed with water and brine and dried. Evaporation and chromatography (EtOAc/hexane 1:4) gave **85** (0.57 g, 60%) as a yellow powder: Rf 0.23 (EtOAC/hexane 1:1); mp >230°C (lit.[113] mp >230°C); ^{1}H NMR ($CDCl_3$) δ.11.17 (1 H, s, NH), 8.22 (1 H, d, J = 7.8 Hz, 8-H), 8.03 (1 H, m, 6-H), 7.48 (1 H, t, J = 7.8 Hz, 7-H), 7.18 (1 H, d, J = 6.2 Hz, 3-H), 6.67 (1 H, s, NH), 6.58 (1 H, d, J = 7.0 Hz, 4-H), 1.54 (9 H, s, Bu^t).



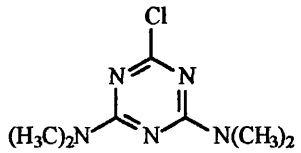
5-Nitroisoquinolin-1-one (86)

Compound **78** (0.5 g, 2.6 mmol) was boiled under reflux for 4 h in 2-methoxyethanol (50ml) saturated with ammonia. The solution was cooled every 30 min and re-saturated with ammonia. The solvent was then removed *in vacuo* until 10 ml remained. The cloudy solution was stored at 4°C overnight. The precipitate was collected, washed (water, ethanol) and dried to yield **86** (0.2 g, 60%) as yellow crystals: mp 247-249°C (lit.[94] mp 250°C); ^{1}H NMR ($CDCl_3$) δ.11.8 (1 H, s, NH), 8.58 (1 H, d, J = 7.7 Hz, 8-H), 8.46 (1 H, d, J = 7.7 Hz, 6-H), 7.66 (1 H, t, J = 7.7 Hz, 7-H), 7.45 (1 H, d, J = 7.7 Hz, 3-H), 6.97 (1 H, d, J = 7.7 Hz, 4-H). MS (FAB +ve) *m/z* 191.0459 (M+H), ($C_9H_7N_2O_3$ requires 191.0456).



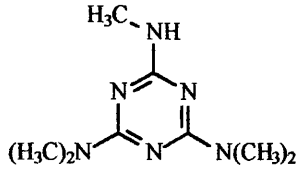
5-(N,N-Bis(1,1-dimethylethoxycarbonyl)amino)isoquinolin-1-one (87)

Et_3N (50 μl) and DMAP (5 mg) were added to **33'** (0.15 g, 0.9 mmol) in DMF (20 ml) and the solution was cooled to 0°C. Di-*tert*-butyl dicarbonate (0.6 g, 2.8 mmol) was added in small quantities over 4 d. The evaporation residue in EtOAc was washed with water and brine and dried. Evaporation and chromatography (EtOAc/Hexane 1:4) gave **87** as a yellow powder (0.2 g, 60%): Rf 0.34 (EtOAC/hexane 1:1); mp 178-180°C; ^{1}H NMR ($CDCl_3$) δ 11.61 (1 H, s, NH), 8.41 (1 H, d, J = 7.8 Hz, 8-H), 7.51 (2 H, m, 6/7-H_2), 7.27 (1 H, d, J = 7.8 Hz, 3-H), 6.56 (1 H, d, J = 7.0 Hz, 4-H), 1.56 (18 H, s, 2 Bu^t); MS (FAB +ve) *m/z* 361.1762 (M+H), ($C_{19}H_{25}N_2O_5$ requires 361.1763).



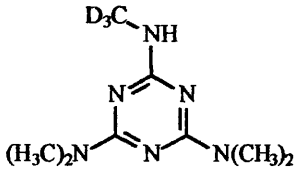
2,4-Bis(dimethylamino)-6-chloro-1,3,5-triazine (89)

2,4,6-Trichloro-1,3,5-triazine **88** (2.0 g, 11 mmol) in acetone (20 ml) was added to crushed ice (50 g) and dimethylamine (22 ml, 43 mmol) was added all at once with stirring. The mixture was stirred at 40°C and then allowed to cool. Filtration and recrystallisation (propanol/water) yielded **89** (1.3 g, 59%) as a white crystalline solid: Rf 0.5 (EtOAc/hexane 1:1); mp 62-64°C (lit.[119] mp 66-68°C); ^{1}H NMR ($CDCl_3$) δ 3.13 (12 H, s, 4 × CH_3)



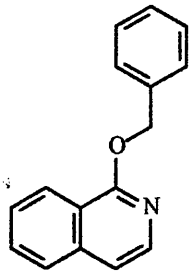
2,4-Bis(dimethylamino)-6-methylamino-1,3,5-triazine (90)

Compound **89** (1.0 g, 4.9 mmol) was added to methylamine (4.3 ml, 49 mmol) in water (7.5 ml). The mixture was boiled under reflux until it became homogeneous. Aqueous NaOH (1 ml, 0.2 g, 4.9 mmol) was added slowly. Reflux was maintained for another 20 minutes. The solution was filtered hot. Evaporation gave an oil which solidified on cooling. The solid was triturated with cold water (50 ml), filtered and dried. Recrystallisation (ethanol/water) gave **90** (0.61 g, 63%) as a white crystalline solid: Rf 0.3 (EtOAc/hexane 1:1); mp 104-105°C (lit.[120] mp 98-103°C); ^{1}H NMR ($CDCl_3$) δ 3.09 (12 H, s, 4 × CH_3), 2.90 (3 H, s, CH_3).



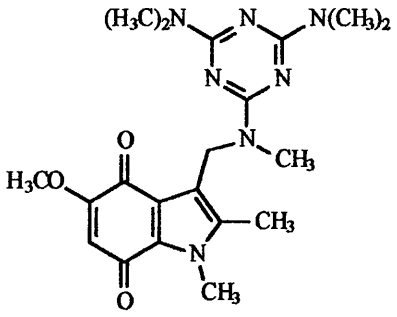
2,4-Bis(dimethylamino)-6-trideuteromethylamino-1,3,5-triazine (91)

Compound **89** (2.0 g, 9.9 mmol) was added to CD_3NH_2.HCl (0.35 g, 4.9 mmol) in water (15 ml). The mixture was boiled under reflux until it became homogeneous. Aqueous NaOH (4 ml, 0.4 g, 9.9 mmol) was added slowly. Reflux was maintained for another 20 minutes. The solution was filtered hot. Evaporation gave an oil which solidified on cooling. The solid was triturated with cold water (50 ml), filtered and dried. Recrystallisation (ethanol/water) gave **91** (1.18 g, 60%) as a white crystalline solid: Rf 0.3 (EtOAc/hexane 1:1); mp 94-95°C; IR 3090, 2933, 2073 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.07 (12 H, s, 4 × CH_3), 4.89 (1 H, s, NH); ^{13}C NMR ($CDCl_3$) δ 166.5 (1-C), 165.4 (3/5-C), 35.8 (CH_3), 26.5 (CD_3, septet, J = 20.8 Hz).



1-Phenylmethoxyisoquinoline (93)

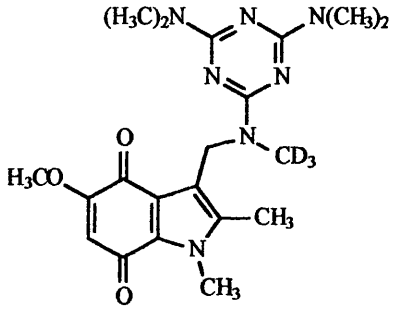
Diethyl azodicarboxylate (0.06 ml, 0.4 mmol) was added dropwise to isoquinolin-1-one **28** (0.05 g, 0.3 mmol) and PPh_3 (0.09 g, 0.4 mmol) in dry THF (20 ml) under dry Ar. The mixture was stirred for 15 min and benzyl alcohol **92** (0.04 ml, 0.3 mmol) was added. The mixture was stirred overnight. Evaporation and chromatography (EtOAc) afforded **93** (31 mg, 39%) as a colourless oil: Rf 0.63 (EtOAc/hexane 1:1); ^{1}H NMR ($CDCl_3$) δ.8.31 (1 H, d, J = 8.5 Hz, isoquinoline 8-H), 8.00 (1 H, d, J = 6.2 Hz, isoquinoline 3-H), 7.7 (1 H, d, J = 8.2 Hz, isoquinoline 5-H), 7.66 (1 H, t, J = 8.2 Hz, isoquinoline 6-H), 7.55 (1 H, t, J = 8.2 Hz, isoquinoline 7-H), 7.36 (5 H, m, Ph), 7.25 (1 H, d, J = 6.2 Hz, isoquinoline 4-H), 5.58 (2 H, s, CH_2); ^{13}C NMR ($CDCl_3$) δ 147.2 (isoquinoline 1-C), 137.8 (Ph 1-C), 137.2 (isoquinoline 8a-C), 130.3 (Ph 2'/6'-CH), 129.4 (isoquinoline 4a-C), 128.3 (Ph 4CH and isoquinoline 6-CH and 7-CH), 127.7 (Ph 3'/5'-CH), 126.5 (isoquinoline 3-CH and 8-CH), 125.9 (isoquinoline 5-CH), 124.1 (isoquinoline 4-CH), 69.4 (CH_2); MS (EI) *m/z* 235.0997 (M) ($C_{16}H_{13}NO$ requires 235.0989).



1,2-Dimethyl 3-{N-[[4,6-bis(dimethylamino)-1,3,5-triazin-2-yl]-N-methyl-amino]methyl}-5-methoxyindole-4,7-dione (94)

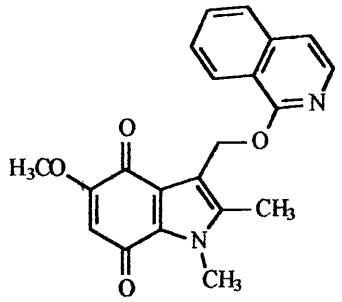
Diethyl azodicarboxylate (0.05 ml, 0.3 mmol) was added dropwise to compound **90** (0.060 g, 0.3 mmol) and PPh_3 (0.080 g, 0.3 mol) in dry THF (15 ml) under dry Ar.

The mixture was stirred for 15 min and **11** (0.070 g, 0.3 mmol) was added. The mixture was stirred overnight. Evaporation and chromatography (EtOAc) afforded **94** (49 mg, 40%) as an orange solid: Rf 0.36 (EtOAc); IR 3090, 2980, 1680, 1690, 1470 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.61 (indole 1 H, s, 6-H), 5.12 (2 H, m, CH_2), 3.88 (3 H, s, CH_3O), 3.81 (3 H, s, indole CH_3N), 3.11 (12 H, s, 2 × $N(CH_3)_2$), 2.94 (3 H, s, melamine CH_3N), 2.21 (3 H, s, indole 2-CH_3); ^{13}C NMR ($CDCl_3$) δ 178.6 (indole 7-CO), 165.2 (indole 4-CO, melamine 2-C), 159.4 (melamine 4/6-C), 137.8 (indole 7a-C and indole 5-C), 122.5 (indole 2-C and indole 3a-C), 120.3 (indole 3-C), 106.6 (6-CH), 56.4 (CH_3O), 39.6 (CH_2), 35.9 ($N(CH_3)_2$), 32.8 (indole N-CH_3), 29.8 (melamine CH_3N), 9.9 (indole 2-CH_3); MS (FAB +ve) *m/z* 414.2253 (M+H) $C_{20}H_{28}N_7O_3$ requires 414.2256).



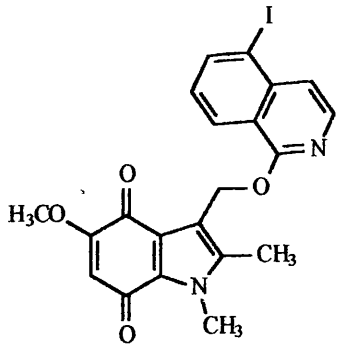
1,2-Dimethyl 3-{N-[[4,6-bis(dimethylamino)-1,3,5-triazin-2-yl]-N-(trideutero)-methylamino]methyl}-5-methoxyindole-4,7-dione (96)

Diethyl azodicarboxylate (0.07 ml, 0.45 mmol) was added dropwise to compound **91** (0.1 g, 0.4 mmol) and PPh_3 (0.12 g, 0.45 mol) in dry THF (15 ml) under dry Ar. The mixture was stirred for 15 min and **11** (0.085 g, 0.4 mmol) was added. The mixture was stirred overnight. Evaporation and chromatography (EtOAc) afforded **96** (35 mg, 20%) as an orange solid: Rf 0.5 (EtOAc); IR 3090, 2980, 2780, 1680, 1690, 1470 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.61 (1 H, s, indole 6-H), 5.14 (2 H, m, CH_2), 3.88 (3 H, s, CH_3O), 3.81 (3 H, s, indole CH_3N), 3.11 (12 H, s, 2 × $N(CH_3)_2$), 2.21 (3 H, s, indole 2-CH_3); ^{13}C NMR ($CDCl_3$) δ 178.6 (indole 7-CO), 165.2 (indole 4-CO, melamine 2-C), 159.8 (melamine 4/6-C), 138.1 (indole 7a-C and indole 5-C), 122.5 (indole 2-C and indole 3a-C), 120.3 (indole 3-C), 106.8 (indole 6-CH), 56.6 (CH_3O), 39.8 (CH_2), 36.1 ($N(CH_3)_2$), 32.8 (indole N-CH_3), 10.1 (indole 2-CH_3), the CD_3 signal was too weak to be observed; MS (FAB +ve) *m/z* 417.2440 (M+H) ($C_{20}{}^2H_3{}^1H_{25}N_7O_3$ requires 417.2441).



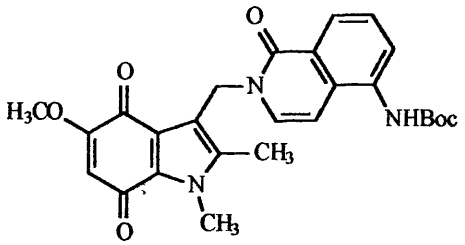
1,2-Dimethyl-3-(isoquinolin-1-yloxymethyl)-5-methoxyindole-4,7-dione (97)

Diethyl azodicarboxylate (0.13 ml, 0.85 mmol) was added dropwise to isoquinolin-1-one **28** (0.06 g, 0.4 mmol) and PPh_3 (0.22 g, 0.85 mmol) in dry THF (20 ml) under dry Ar. The mixture was stirred for 15 min and **11** (0.1 g, 0.4 mmol) was added. The mixture was stirred overnight. Evaporation and chromatography (EtOAc) afforded **97** (59 mg, 40%) as a red powder: Rf 0.47 (EtOAc); mp >230°C; IR 3042, 2991, 1725, 1694/1671.7, 1465/1399 cm^{-1}; ^1H NMR ($CDCl_3$) δ. 8.17 (1 H, d, J = 8.6 Hz, isoquinoline 8-H), 8.00 (1 H, d, J = 5.8 Hz, isoquinoline 3-H), 7.70 (1 H, d, J = 8.2 Hz, isoquinoline 5-H), 7.61 (1 H, td, J = 8.2, 1.1 Hz, isoquinoline 6-H), 7.45 (1 H, td, J = 8.2, 1.1 Hz, isoquinoline 7-H), 7.20 (1 H, d, J = 5.8 Hz, isoquinoline 4-H), 5.72 (2 H, s, CH_2), 5.62 (1 H, s, indole 6-H), 3.89 (3 H, s, CH_3O), 3.80 (3 H, s, CH_3N), 2.38 (3 H, s, indole 2-CH_3); ^{13}C NMR ($CDCl_3$) δ 178.9 (indole 7-CO), 177.6 (indole 4-CO), 160.3 (indole 5-C), 159.7 (isoquinoline 1-C), 142.4 (isoquinoline 8-CH), 139.6 (isoquinoline 3-CH), 138.0 (isoquinoline 4a-C), 137.9 (isoquinoline 6-CH), 130.3 (indole 2-C), 129.0 (indole 7a-C), 126.4 (isoquinoline 7-CH), 125.9 (isoquinoline 5-CH), 124.4 (isoquinoline 8a-C), 122.0 (indole 3a-C), 119.8 (indole 3-C), 117.3 (isoquinoline 4-CH), 114.8 (indole 6-CH), 56.4 (CH_3O), 53.4 (CH_2), 50.8 (CH_3N), 9.8 (indole 2-CH_3); MS (EI) *m/z* 363.1346 (M) ($C_{21}H_{18}N_2O_4$ requires 363.1344).



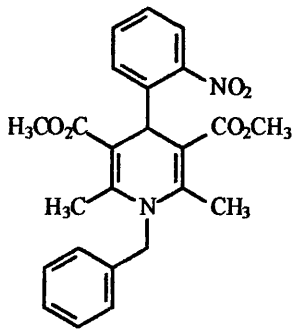
1,2-Dimethyl-3-(5-iodoisoquinolin-1-yloxymethyl)-5-methoxyindole-4,7-dione (99)

Diethyl azodicarboxylate (0.12 ml, 0.7 mmol) was added dropwise to 5-iodo-isoquinolin-1-one **34** (0.1 g, 0.4 mmol) and PPh_3 (0.19 g, 0.7 mmol) in dry THF (20 ml) under dry Ar. The mixture was stirred for 15 min and **11** (0.085 g, 0.4 mmol) was added. The mixture was stirred overnight. Evaporation and chromatography (EtOAc) afforded **99** (69 mg, 39%) as a purple powder Rf 0.46 (EtOAc); mp >230°C; IR 3096, 2923, 1702, 1470, 1380 cm^{-1}; ^1H NMR ($CDCl_3$) δ. 8.20 (1 H, d, J = 8.3 Hz, isoquinoline 8-H), 8.15 (1 H, dd, J = 7.4, 1.0 Hz, isoquinoline 6-H), 8.08 (1 H, d, J = 6.1 Hz, isoquinoline 3-H), 7.40 (1 H, d, J = 6.1 Hz, isoquinoline 4-H), 7.16 (1 H, t, J = 8.0 Hz, isoquinoline 7-H), 5.71 (2 H, s, CH_2), 5.62 (H, s, indole 6-H), 3.89 (3 H, s, CH_3O), 3.80 (3 H, s, CH_3N), 2.37 (3 H, s, indole 2-CH_3); ^{13}C NMR ($CDCl_3$) δ 178.9 (indole 7-CO), 177.6 (indole 4-CO), 160.5 (indole 5-C), 159.7 (isoquinoline 1-C), 141.1 (isoquinoline 4a-C), 139.5 (isoquinoline 3-CH), 138.0 (indole 7a-C), 134.5 (isoquinoline 6-CH), 128.9 (indole 2-C), 127.5 (isoquinoline 8-CH), 125.1 (isoquinoline 7-CH), 120.6 (indole 3a-C and isoquinoline 8a-C), 118.5 (indole 3-C), 117.0 (indole 4-CH), 106.6 (isoquinoline 4-CH and 5-C), 58.6 (CH_3O), 56.4 (CH_2), 32.4 (CH_3N), 9.86 (indole 2-CH_3); MS (FAB +ve) *m/z* 489.0309 (M+H) ($C_{21}H_{18}IN_2O_4$ requires 489.0311).



1,2-Dimethyl-3-[5-(1,1-dimethylethoxycarbonylamino)-1-oxoisoquinolin-2-ylmethyl]-5-methoxyindole-4,7-dione (100)

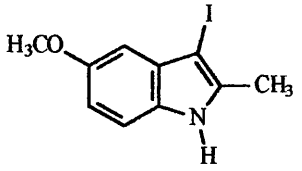
Diethyl azodicarboxylate (**0.12** ml, 0.8 mmol) was added dropwise to **85** (0.10 g, 0.4 mmol) and PPh_3 (0.20 g, 0.8 mmol) in dry THF (20 ml) under dry Ar. The mixture was stirred for 15 min and **11** (0.090 g, 0.4 mmol) was added. The mixture was stirred overnight. Evaporation and chromatography (EtOAc) afforded **100** (22 mg, 12%) as an orange solid: Rf 0.51 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 8.21 (1H, d, J = 8.0 Hz, isoquinoline 8-H), 7.47 (2 H, m, isoquinoline 3-H and 6-H), 7.27 (1 H, t, J = 8.0 Hz, isoquinoline 7-H), 6.57 (1 H, d, J = 8.0 Hz, isoquinoline 4-H), 5.62 (1 H, s, indole 6-H), 5.25 (2 H, s, CH_2), 3.88 (3 H, s, CH_3O), 3.81 (3 H, s, CH_3N), 2.47 (3 H, s, indole 2-CH_3), 1.54 (9 H, s, Bu^t).



Dimethyl 1-benzyl-2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (103)

$LiN(SiMe_3)_2$ (1.0 M in THF) (7.8 ml, 7.8 mmol) and nifedipine **101** (1.35 g, 3.9 mmol) were stirred together in DMF (10 ml) for 2 h under dry Ar. Benzyl chloride (0.5 g, 3.9 mmol) was added followed by NaI (5 mg). The mixture was stirred for 2 d. The evaporation residue in EtOAc was washed with water and brine and dried. Evaporation and chromatography (hexane/EtOAc 1:1) yielded **103** (0.68 g, 40%) as a

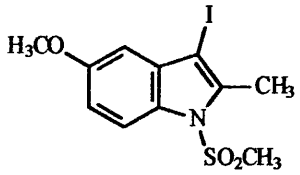
yellow glass: Rf 0.43 (EtOAc/hexane 1:1); ^{1}H NMR ($CDCl_3$) δ 7.65-7.20 (9 H, m, Ar, $ArNO_2$), 5.70 (1 H, s, 4-H), 4.93 (2 H, s, CH_2), 3.63 (6 H, s, 2 × OCH_3), 2.4 (6 H, s, 2 × CH_3); MS (FAB +ve) *m/z* 437 (M+H).



3-Iodo-5-methoxy-2-methylindole (109)

Method 1: ICl (0.71 ml, 6.2 mmol) and 1,4-dioxane (0.8 ml) were added to **57** (0.1 g, 6.2 mmol) in pyridine (5 ml) at 0°C. The solution was stirred overnight. The solvents were evaporated. The residue, in CH_2Cl_2,was washed with aq. Na_2CO_3 (10%), aq. HCl (2 M), aq. $NaHCO_3$ (saturated) and water and was dried. Evaporation gave **109** (0.089 g, 50%) as a buff solid: Rf 0.27 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 8.52 (1 H, s, NH), 7.99 (1 H, s, 4-H), 7.18 (1 H, d, J = 9.3 Hz, 7-H), 6.73 (1 H, m, 6-H), 3.80 (3 H, s, CH_3O), 2.37 (3 H, s, 2-CH_3).

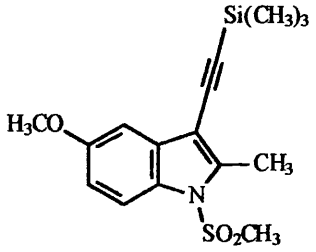
Method 2: KOH (1.3 g, 23 mmol) was added to **57** (1.0 g, 6.2 mmol) in DMF (4.0 ml) and the mixture was stirred 5 min. I_2 (1.57 g, 6.2 mmol) in DMF (4.0 ml) was added dropwise. The mixture was stirred for 10 min and was poured into a mixture of $Na_2S_2O_5$, (0.91 g, 4.8 mmol) and 25 % aqueous NH_3 (8.53 ml) in water (150 ml). The precipitate was filtered off to give **109** (1.07 g, 60%) as a buff solid which was used immediately without further purification because of its instability. (data above).



3-Iodo-5-methoxy-2-methyl-1-methylsulfonylindole (110)

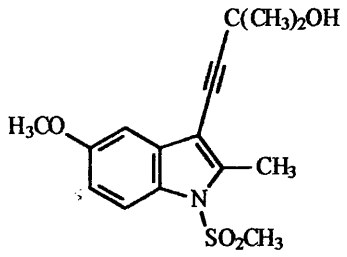
$MeSO_2Cl$ (1.72 g,15 mmol) in benzene (15ml) was added dropwise under vigorous stirring to **109** (1.0 g, 3.5 mmol), Bu_4NBr (0.2 g, 0.62 mmol), NaOH (5.0 g), benzene

(15 ml) and water (25 ml). Stirring was continued for 1 h, the organic layer was washed with water and dried. Evaporation, chromatography (toluene) and recrystallisation yielded **110** (0.24 g, 19%) as an off-white solid: Rf 0.7 (EtOAc); mp 146-147°C; IR 3090, 2970, 1600, 1470 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.85 (1 H, d, J = 9.1 Hz, 7-H), 6.95 (1 H, dd, J = 9.1, 2.5 Hz, 6-H), 6.84 (1 H, d, J = 2.5 Hz, 4-H), 3.93 (3 H, s, CH_3O), 3.00 (3 H, s, SO_2CH_3), 2.68 (3 H, s, 2-CH_3); ^{13}C NMR ($CDCl_3$) δ 157.3 (5-C), 138.0 (2-C), 132.8 (3a-C), 130.4 (7a-C), 115.1 (7-CH), 114.1 (6-CH), 104.1 (4-CH), 73.1 (3-C), 55.7 (OCH_3), 40.6 (SCH_3), 16.4 (CH_3); MS (FAB+ve) *m/z* 365.9678 (M+H) ($C_{11}H_{13}INO_3S$ requires 365.9660); Anal. C 36.60%, H 3.39%, N 3.86% ($C_{11}H_{12}INO_3S$ requires C 36.26%, H 3.29%, N 3.84%).



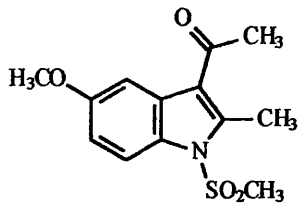
5-Methoxy-2-methyl-1-(methylsulfonyl)-3-(2-trimethylsilylethynyl)indole (111)

A mixture of **110** (0.50 g, 1.3 mmol), trimethylsilylethyne (0.14 g, 1.4 mmol), $Pd(PPh_3)_2Cl_2$ (32 mg, 0.045 mmol), CuI (16 mg, 0.084 mmol) and DMF (10 ml) was stirred under Ar for 10 min and triethylamine (1.22 ml, 8.7 mmol) was added. The mixture was stirred for 3 d. The mixture was diluted with water (20 ml) and extracted with diethyl ether (3 × 10 ml). The ether extracts were washed with water and dried. Evaporation, chromatography (EtOAc/hexane 1:1) and recrystallisation (pentane) yielded **111** (0.25 g, 60%) as a light yellow solid: Rf 0.6 (EtOAc/hexane 1:1); mp 98-102°C; IR (KBr) 3090, 2970, 2260, 1610, 1470, 1260, 850, 780 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.83 (1 H, d, J = 9.1 Hz, 7-H), 7.05 (1 H, d, J = 2.6 Hz, 4-H), 6.94 (1 H, dd, J = 9.1, 2.6 Hz, 6-H), 3.88 (3 H, s, CH_3O), 3.00 (3 H, s, SO_2CH_3), 2.68 (3 H, s, 2-CH_3), 0.30 (9 H, s, $Si(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ 157.2 (5-C), 142.2.0 (2-C), 130.9 (7a-C), 130.0 (3a-C), 115.0 (7-CH), 113.7 (6-CH), 105.6 (alkyne 1-C), 102.8 (4-CH), 101.6 (3-C), 96.4 (alkyne 2-C), 56.3 (CH_3O), 41.0 (SO_2CH_3), 14.0 (CH_3), -0.1 ($SiCH_3$); MS (FAB +ve) *m/z* 336.1067 (M+H) ($C_{16}H_{22}NO_3SSi$ requires 336.1089).



5-Methoxy-2-methyl-1-(methylsulfonyl)-3-(3-hydroxy-3-methyl-1-butynyl)indole (112)

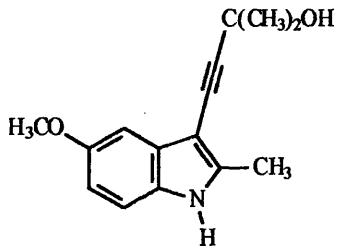
A mixture of **110** (0.08 g, 0.21 mmol), 2-methyl-3-butyn-2-ol (0.02 g, 0.23 mmol), $Pd(PPh_3)_2Cl_2$ (32 mg, 0.045 mmol), CuI (16 mg, 0.084 mmol) and DMF (10 ml) was stirred under Ar for 10 min and triethylamine (0.18 ml, 1.3 mmol) was added. The mixture was stirred for 3 d. The mixture was diluted with water (20 ml) and extracted with diethyl ether (3 × 10 ml). The ether extracts were washed with water and dried. Evaporation, chromatography (EtOAc/hexane 1:1) and recrystallisation (pentane) yielded **112** (0.04 g, 60%) as an off-white powder: Rf 0.5 (EtOAc/hexane 1:1); mp 110-115°C; IR (KBr) 3090, 2970, 2260, 1610, 1470, 1260, 850, 780 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.85 (1 H, d, J = 8.9 Hz, 7-H), 7.01 (1 H, d, J = 2.3 Hz, 4-H), 6.90 (1 H, dd, J = 8.9, 2.3 Hz, 6-H), 3.88 (3 H, s, CH_3O), 3.01 (3 H, s, SO_2CH_3), 2.69 (3H, s, 2-CH_3), 1.69 (6 H, s, CH_3); ^{13}C NMR ($CDCl_3$) δ 156.9 (5-C), 141.1 (2-C), 130.6 (3a-C), 129.8 (7a-C), 114.7 (7-CH), 113.0 (6-CH), 104.6 (alkyne 1-C), 102.2 (4-CH), 100.7 (3-C), 73.3 (alkyne 2-C), 65.8 (alkyne 3-C), 55.7 (OCH_3), 40.7 (SO_2CH_3), 31.7 ($(CH_3)_2$), 14.2 (CH_3); MS (FAB +ve) *m/z* 322.1081 (M+H) ($C_{16}H_{20}NO_3SSi$ requires 322.1113).



3-Acetyl-5-methoxy-2-methyl-1-methylsulfonylindole (113)

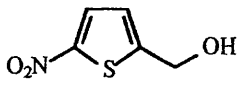
Compound **111** (0.1 g, 0.39 mmol) in methanolic KOH (5 % w/v 10 ml) was boiled under reflux for 20 h. It was cooled, diluted with water (20 ml) and extracted with ether (3 × 10 ml). The ether fractions were washed with water and dried. Evaporation

yielded **113** (0.056 g, 71%) as a yellow powder: Rf 0.1 (EtOAc/hexane 1:1); mp 219-221°C; IR (KBr)) 3450, 3090, 2970, 1860, 1650, 1600, 1470 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.40 (1 H, s, NH), 7.60 (1 H, d, J = 2.4 Hz, 4-H), 7.21 (1 H, d, J = 8.7 Hz, 7-H), 6.85 (1 H, dd, J = 8.7, 2.4 Hz, 6-H), 3.88 (3 H, s, CH_3O), 2.73 (3 H, s, CH_3CO), 2.71 (3 H, s, 2-CH_3); ^{13}C NMR ($CDCl_3$) δ 194.0 (CO), 155.6 (5-C), 144.5 (2-C), 129.9 (7a-C), 128.0 (3a-C), 114.2 (7-CH), 111.6 (3-C), 111.3 (6-CH), 103.7 (4-CH), 55.8 (OCH_3), 30.9 ($COCH_3$), 15.6 (2-CH_3); MS (FAB +ve) *m/z* 204.1028 (M+H) ($C_{12}H_{14}NO_2$ requires 204.1024).



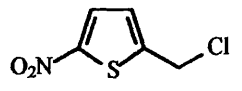
5-Methoxy-2-methyl-3-(3-hydroxy-3-methylbut-1-ynyl)indole (117)

Compound **112**. (0.07g, 0.22 mmol) in dry THF (10 ml) was boiled under reflux with Bu_4NF (0.05g, 0.22 mmol) under Ar overnight. The solvent was evaporated. Water (5 ml) was added. The mixture was extracted with diethyl ether and dried. Evaporation and recrystallisation (acetone/hexane) gave **117** (53 mg, 50%) as a yellow solid: Rf 0.2 (EtOAc); IR (KBr) 3276, 2982, 1623, 1453-1366, 1210, 1168 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.16 (1 H, br s, NH), 7.15 (1 H, d, J = 7.8 Hz, 7-H), 7.03 (1 H, d, J = 2.7 Hz, 4-H), 6.79 (1 H, dd, J = 2.7, 7.8 Hz, 6-H), 3.88 (3 H, s, CH_3O), 2.33 (3 H, s, 2-CH_3), 1.71 (6 H, s, 2 × CH_3); ^{13}C NMR ($CDCl_3$) δ 156.3 (5-C), 134.0 (2-C), 129.8 (7a-C), 127.8 (3a-C), 114.5 (7-CH), 111.6 (6-CH), 103.3 (alkyne 1-C), 100.7 (4-CH), 97.9 (3-C), 70.1 (alkyne 2-C), 65.0 (alkyne 3-C), 55.3 (CH_3O), 30.9 ($(CH_3)_2$), 11.7 (2-CH_3); MS (FAB +ve) *m/z* 244.1334 (M+H), ($C_{15}H_{18}NO_2$ requires 244.1337).



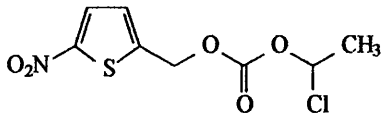
(5-Nitro-2-thienyl)methanol (119)

$NaBH_4$ (0.96 mg, 2.5 mmol) was added slowly to 5-nitro-2-thienaldehyde **118** (0.1 g, 0.63 mmol) in absolute MeOH (5 ml) at 0°C. The mixture was stirred at 20°C for 20 min. Water was added (15 ml), and the mixture was extracted with diethyl ether (3 × 15 ml). The ether extracts were washed with water and brine and the solvent was evaporated. Chromatography (EtOAc/hexane 1:1) gave **119** (0.071 g, 70%) as a yellow oil: (lit.[148] $bp_{3\ mmHg}$ 130-133°C); ^1H NMR ($CDCl_3$) δ 7.81 (1 H, d, J = 4.1 Hz, 4-H), 6.94 (1 H, d, J = 4.1 Hz, 3-H), 4.88 (2 H, s, CH_2), 2.03 (1 H, s, OH).



2-Chloromethyl-5-nitrothiophene (120)

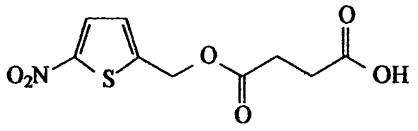
Thionyl chloride (0.091 ml, 1.25 mmol) was added to **119** (0.1 g, 0.63 mmol) at 0-5°C over 4 h. The mixture was stirred at 5°C for a further hour, water was added (15 ml), and the mixture was extracted with diethyl ether (3 × 15 ml). The ether extracts were washed with water and brine and the solvent was evaporated. Chromatography (EtOAc/hexane 1:1) gave **119** (0.052 g, 52%) as a yellow oil: (lit.[148] $bp_{3\ mm}$ 105-107°C); ^1H NMR (DMSO) δ 7.31 (1 H, d, J = 4.1 Hz, 4-H), 7.07 (1 H, d, J = 4.1 Hz, 3-H), 4.72 (2 H, s, CH_2).



1-Chloroethyl (5-nitro-2-thienyl)methyl carbonate (124)

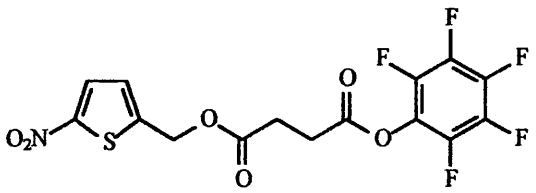
Pyridine (5 ml) was added to **119** (0.3 g, 1.9 mmol) in dry THF (10 ml), and the mixture was stirred for 15 min. 1-Chloroethyl chloroformate (0.22 mL, 1.9 mmol) was

added dropwise and the mixture was stirred overnight. The evaporation residue in CH_2Cl_2 was washed with saturated aqueous K_2CO_3 and water and dried. Evaporation and chromatography (hexane/EtOAc 1:1) yielded **124** (0.27 g, 54%) as a yellow oil: Rf 0.5 (EtOAc/hexane 1:1); ^{1}H NMR ($CDCl_3$) δ 7.82 (1 H, d, J = 4.3 Hz, 4-H), 7.11 (1 H, d, J = 4.3 Hz, 3-H), 6.44 (1 H, q, J = 5.8 Hz, CH), 5.37 (1 H, d, J = 12.5 Hz) and 5.31 (1 H, d, J = 12.5 Hz) (CH_2), 1.82 (3 H, d, J = 5.8 Hz, CH_3).



4-(5-Nitro-2-thienylmethoxy)-4-oxobutanoic acid (126)

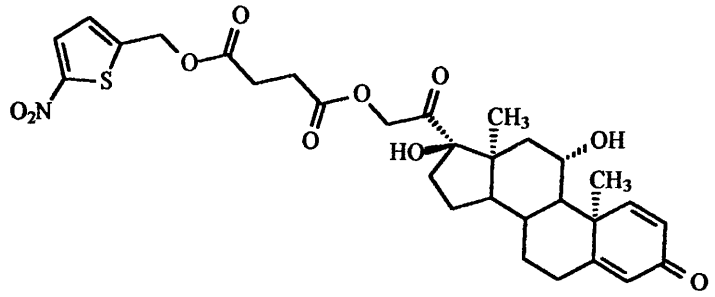
Compound **119** (1.0 g, 6.3 mmol) was added to a mixture of succinic anhydride (0.63 g, 6.3 mmol) and DMAP (5 mg) in pyridine (5 ml) and the mixture was stirred for 8 h at 50 °C. After cooling and evaporation, the residue in EtOAc, was washed with water and dried. Evaporation and chromatography (EtOAc) gave **126** (1.23 g, 76%) as an off-white solid: Rf 0.4 (EtOAc); mp 93-95°C; IR 3090, 2970, 1730, 1690, 1470 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.25 (1 H, d, J = 5.0 Hz, 4'-H), 7.03 (1 H, d, J = 5.0 Hz, 3'-H), 5.27 (2 H, s, CH_2), 2.70 (4 H, m, 2 × CH_2); ^{13}C NMR ($CDCl_3$) δ 173.9 (1-CO), 171.9 (4-CO), 146.3 (5' and 2'-C), 128.3 (4'-CH), 126.7 (3'-CH), 60.4 (CH_2), 28.9 (3/2-CH_2); MS (FAB +ve) *m/z* 260.0230 (M+H) ($C_9H_{10}NO_6S$ requires 260.0228). Anal. C 41.20%, H 3.45%, N 5.61% ($C_9H_{10}NO_6S$ requires C 41.69%, H 3.47%, N 5.40%).



1-(5-Nitro-2-thienylmethyl)-4-pentafluorophenyl butanedioate (128)

Compound **126** (0.22 g, 0.85 mmol) was stirred in EtOAc (5 ml) at 0°C for 15 min. Pentafluorophenol (0.15 g, 0.85 mmol) in EtOAc (3 ml) was added, followed by 1,3-dicyclohexylcarbodiimide (0.17 g, 0.85 mmol) in EtOAc (3 ml). The mixture was

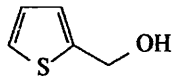
stirred for 5 h and was left at 4°C overnight. Filtration and evaporation gave **128** (0.35 g, 98%) as a buff oil: Rf 0.6 (EtOAc); IR 3090, 2970, 2260, 1770, 1730, 1470 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.81 (1 H, d, J = 4.2 Hz, 4-H), 7.04 (1 H, d, J = 4.2 Hz, 3-H), 5.29 (2 H, s, CH_2O), 3.04 (2 H, t, J = 6.2 Hz, $COCH_2$), 2.84 (2 H, t, J = 6.2 Hz, CH_2CO); ^{13}C NMR ($CDCl_3$) δ 170.8 (ester CO), 168.1 (ester CO), 145.3 (thiophene 2 and 5-C), 128.1 (thiophene 4-CH), 126.9 (thiophene 3-CH), 60.8 (CH_2), 28.7 and 28.2 (CH_2CH_2); Owing to multiple C-F coupling and to the quaternary nature of these carbons, the signals for the C_6F_5 carbons were too weak to be observed; MS (FAB +ve) *m/z* 426.0078 (M+H) ($C_{15}H_9F_5NO_6S$ requires 426.0070).



(5-Nitro-2-thienyl)methyl prednisolon-21-yl butanedioate (129)

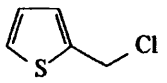
Compound **128** (0.22 g, 0.52 mmol) was stirred with prednisolone (0.18 g, 0.52 mmol) and DMAP in dry DMF (10 ml) for 2 days. The evaporation residue in CH_2Cl_2 was washed with aq. HCl (1 M), water and was dried. Evaporation yielded **129** as a yellow-orange glass (0.233 g, 75%): IR (KBr) 3450, 3090, 2970, 1740, 1650, 1600, 1470 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.81 (1 H, d, J = 4.3 Hz, thiophene 4-H), 7.29 (1 H, d, J = 10.5 Hz, steroid 2-H), 7.04 (1 H, d, J = 4.3 Hz, thiophene 3'-H), 6.27 (1 H, d, J = 10.5 Hz, steroid 1-H), 6.00 (1 H, s, steroid 4-H), 5.28 (2 H, s, CH_2), 5.00 (1 H, d, J = 17.5 Hz) and 4.90 (1 H, d, J = 17.5 Hz) (steroid 21-H_2), 4.49 (1 H, s, steroid 11-H), 2.80 (4 H, m, butanedioate CH_2CH_2), 2.6-2.0 (4 H, m, steroid 6 and 16-H_4), 1.95-1.45 (5 H, m, steroid 9, 12 and 15-H_5), 1.45 (3 H, s, steroid 19-CH_3), 1.2-1.0 (5 H, m, steroid 7, 8 and 14-H_5), 0.95 (3 H, s, steroid 18-CH_3); ^{13}C NMR ($CDCl_3$) δ 206.1 (steroid 21-CO), 186.1 (steroid 3-CO), 172.0 (2 × ester CO), 171.0 (steroid 5-C), 157.0 (steroid 1-CH), 148.0 (thiophene 5-C and 2-C), 125.0 (thiophene 4-CH and 3-CH and steroid 2-CH), 122.2 (steroid 4-CH), 89.5 (steroid 17-C), 69.9 (steroid 11-CH), 68.3 (steroid 21-CH_2), 67.2 (CH_2), 55.4 (steroid 9-CH), 51.4 (steroid 14-CH), 47

(steroid 13 and 10-C), 39.5 (steroid 12-CH_2), 34.6 (steroid 16-CH_2), 34.1 (steroid 7-CH_2), 32.0 (steroid 6-CH_2), 31.3 (butanedioate 2 and 3-CH_2), 23.9 (steroid 15-CH_2), 21.1 (steroid 19-CH_3), 17.0 (steroid 18-CH_3); MS (FAB +ve) *m/z* 602.2084 (M+H) ($C_{30}H_{36}NO_{10}S$ requires 602.2059).



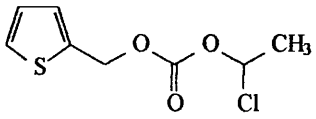
2-Hydroxymethylthiophene (132)

2-Thiophene carboxaldehyde **130** (0.83 ml, 8.9 mmol), MeOH (5 ml) and formaldehyde **131** (1.33 ml, 17.8 mmol) were heated together at 65°C aq. NaOH (2.0 g in 2 ml of H_2O) was added. Heating was continued for 30 min and the solution was refluxed for a short time. The mixture was extracted with benzene and dried. Evaporation and chromatography (EtOAc/hexane 1:1) yielded **132** (0.48 g, 48%) as an orange oil: Rf 0.57 (EtOAc); (lit.[176] $bp_{3\ mm}$ 130-133°C); ^1H NMR (DMSO) δ 7.50 (1 H, dd, J = 5.0, 1.1 Hz, 5-H), 7.10 (1 H, d, J= 3.5, 1.1 Hz, 3-H), 7.00 (1 H, dd, J = 3.5, 5.0 Hz, 4-H), 5.20 (2 H, s, CH_2), 2.03 (1 H, s, OH).



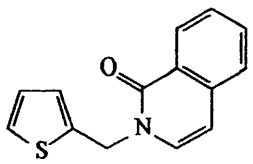
2-Chloromethylthiophene (133)

To **132** (0.5 g, 4.4 mmol) in CH_2Cl_2 (5 ml) at 0°C was added $SOCl_2$ (0.64 ml, 8.8 mmol) followed by the addition of pyridine (0.42 ml, 5.3 mmol). The mixture was stirred at 20°C for 30 min and concentrated. The brown oil was filtered through a silica gel pad to yield **133** (0.37g, 64%) as an orange oil: Rf 0.65 (EtOAc/hexane 1:1); (lit.[177] $bp_{3\ mm}$ 105-107°C); ^1H NMR ($CDCl_3$) δ 7.31 (1 H, dd, J = 5.1, 1.1 Hz, 5-H), 7.09 (1 H, d, J = 3.5 Hz, 3-H), 6.95 (1 H, dd, J = 5.1, 3.5 Hz, 4-H), 4.72 (2 H, s, CH_2).



1-Chloroethyl 2-thienylmethyl carbonate (134)

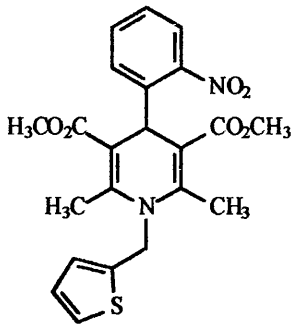
Pyridine (5 ml) was added to **132** (0.2 g, 1.7 mmol) in dry THF (10 ml), and the mixture was stirred for 15 min. 1-Chloroethyl chloroformate (0.18 ml, 1.7 mmol) was added dropwise and the mixture was stirred overnight. The evaporation residue in CH_2Cl_2 was washed with saturated aqueous K_2CO_3 and water and dried. Evaporation and chromatography (hexane/EtOAc 1:1) yielded **134** (0.24 g, 63%) as a yellow oil: Rf 0.66 (EtOAc/hexane 1:1); ^{1}H NMR ($CDCl_3$) δ 7.31 (1 H, d, J = 5.1 Hz, 5-H), 7.09 (1 H, d, J = 3.5 Hz, 3-H), 6.95 (1 H, dd, J = 5.1, 3.5 Hz 4-H), 6.44 (1 H, q, J = 5.8 Hz, CH), 5.40 (1 H, d, J = 12.5 Hz) and 5.33 (1 H, d, J = 12.5 Hz) (CH_2), 1.82 (3 H, d, J = 5.8 Hz, CH_3).



2-(2-Thienylmethyl)isoquinolin-1-one (135)

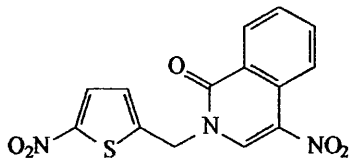
$LiN(SiMe_3)_2$ (2.8 ml, 2.8 mmol) and isoquinolin-1-one (0.2 g, 1.4 mmol) were stirred together in DMF (10 ml) for 2 h under dry Ar. Compound **133** (0.27 g, 1.4 mmol) was added together with NaI (5 mg). The mixture was stirred for 2 d. The evaporation residue in EtOAc was washed with water and brine and dried. Evaporation and chromatography (hexane/EtOAc 1:1) afforded **135** (0.15 mg, 45%) as a light orange glass: Rf 0.6 (EtOAc); IR (KBr) 3090, 2950, 1640, 1600, 1610, 1430 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.45 (1 H, d, J = 7.5 Hz, isoquinoline 8-H), 7.62 (1 H, t, J = 7.4 Hz, isoquinoline 6-H), 7.50 (2 H, m, isoquinoline 3-H and 7-H), 7.24 (1 H, dd, J = 5.2, 1.4 Hz, thiophene 5-H), 7.14 (1 H, d, J = 7.4 Hz, isoquinoline 5-H), 7.11 (1 H, d, J = 3.5 Hz, thiophene 3-H), 6.95 (1 H, dd, J = 5.2, 3.5 Hz, thiophene 4-H), 6.49 (1 H, d, J = 7.3 Hz, isoquinoline 4-H), 5.34 (2 H, s, CH_2); ^{13}C NMR ($CDCl_3$) δ 161.9 (CO), 138.8 (thiophene 2-C), 136.9 (isoquinoline 3-CH), 132.3 (isoquinoline 4a-C), 130.6 (iso-

quinoline 6-CH), 128.0 (thiophene 4-CH and 5-CH), 127.3 (isoquinoline 8-CH), 126.9 (isoquinoline 5-CH), 126.8 (isoquinoline 7-CH), 126.1 (isoquinoline 8a-C), 125.9 (isoquinoline 3-CH), 106.6 (isoquinoline 4-CH), 46.5 (CH_2); MS (FAB +ve) *m/z* 242.0635 (M+H) ($C_{14}H_{12}NOS$ requires 242.0639).



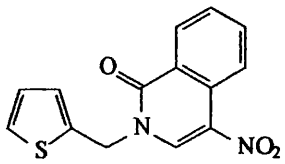
Dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1-(2-thienylmethyl)-1,4-dihydro-pyridine-3,5-dicarboxylate (136)

$LiN(SiMe_3)_2$ (1.0 M in THF) (3.0 ml, 3.0 mmol) and nifedipine **101** (0.52 g, 1.5 mmol) were stirred together in DMF (10 ml) for 2 h under dry Ar. **133** (0.2 g, 1.5 mmol) was added followed by NaI. The mixture was stirred for 2 d. The evaporation residue in EtOAc was washed with water and brine and dried. Evaporation and chromatography (hexane/EtOAc 1:1) yielded the **136** (0.060 g, 10%) as a light orange glass: Rf 0.4 (EtOAc/hexane 1:1); IR 3090, 2970, 1730, 1690, 1470 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.65-7.00 (7 H, m, thiophene, $ArNO_2$), 5.64 (1 H, s, 4-H), 5.06 (2 H, s, CH_2), 3.61 (6 H, s, 2 × OCH_3), 2.53 (6 H, s, 2 × CH_3); ^{13}C NMR ($CDCl_3$) δ 168.1 (2 × CO), 149.7 (2'-C), 146.9 (2/6-C), 140.9 (2-C), 137.1 (5'-CH), 136.2 (1'-C), 132.4 (5-CH/4'-CH), 128.2 (4-CH), 127.3 (3'-CH), 126.3 (3-CH), 124.7 (3/5-C), 52.1 (CH_2), 50.9 (OCH_3), 44.1 (4-CH), 16.9 (CH_3); MS (FAB -ve) *m/z* 441.1118 (M-H) ($C_{22}H_{21}N_2O_6S$ requires 441.1120).



4-Nitro-2-[(5-nitro-2-thienyl)methyl]isoquinolin-1-one (137)

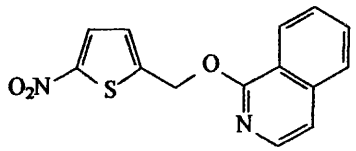
Conc. HNO_3 (0.05 ml, 0.8 mmol) was added to **135** (0.050 g, 0.2 mmol) in TFA (1 ml) at -10°C and the mixture was stirred at 20°C for 16 h. The pH of the mixture was adjusted to 5 with 2 M aq. NaOH and the mixture was extracted with EtOAc. The organic layer was washed with water, 10% aq. $NaHCO_3$, brine and dried. Evaporation and chromatography (EtOAc/hexane 1:1) afforded **137** (28 mg, 42%) as an off-white solid: Rf 0.3 (EtOAc/hexane 1:1); mp 70-73°C; IR 3090, 2963, 1638, 1598, 1570, 1392 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.70 (2 H, m, isoquinoline 8-H and 3-H), 8.51 (1 H, d, J = 7.5 Hz, isoquinoline 5-H), 7.89 (1 H, t, J = 7.5 Hz, isoquinoline 6-H), 7.80 (1 H, d, J = 4.1 Hz, thiophene 4-H), 7.65 (1 H, t, J = 7.5 Hz, isoquinoline 7-H), 7.18 (1 H, d, J = 4.1 Hz, thiophene 3-H), 5.4 (2 H, s, CH_2); ^{13}C NMR ($CDCl_3$) δ 183.3 (CO), 160.9 (thiophene 2-C and 5-C), 143.9 (isoquinoline 3-CH), 135.4 (isoquinoline 6-CH and 4a-C), 134.6 (thiophene 4'-CH and isoquinoline 8a-C), 130.1 (isoquinoline 7-CH), 128.8 (isoquinoline 8-CH), 128.2 (isoquinoline 5-CH), 127.3 (isoquinoline 4-C), 123.8 (thiophene 3-CH), 48.6 (CH_2); MS (FAB +ve) *m/z* 332.0340 (M+H) ($C_{14}H_{10}O_5N_3S$ requires 332.0341).



4-Nitro-2-(2-thienylmethyl)isoquinolin-1-one (138)

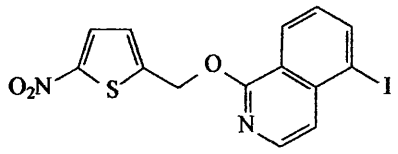
Conc. HNO_3 (65 μl, 1.0 mmol) was added to a mixture of **135** (0.05 g, 0.2 mmol) in Ac_2O (94 μl, 1.0 mmol) and AcOH (5 ml) at -10°C. The mixture was stirred for 40 min at -10°C. Water was added and the solution was extracted with EtOAc. The organic layer was washed with 10% aq. Na_2CO_3 and dried. Evaporation and chromatography (EtOAc/hexane 1:1) yielded **138** (20 mg, 40%) as an off white solid:

Rf 0.5 (EtOAc/hexane 1:1); mp 75-77°C; IR 3090, 2925, 1670, 1630, 1508, 1408 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.67 (2 H, m, isoquinoline 8-H and 3-H), 8.53 (1 H, d, J = 6.8 Hz, isoquinoline 5-H), 7.85 (1 H, t, J = 7.2 Hz, isoquinoline 6-H), 7.64 (1 H, t, J = 7.2 Hz, isoquinoline 7-H), 7.33 (1 H, d, J = 4.6 Hz, thiophene 5-H), 7.22 (1 H, d, J = 3.8 Hz, thiophene 3-H), 7.01 (1 H, m, thiophene 4-H), 5.43 (2 H, s, CH_2); ^{13}C NMR ($CDCl_3$) δ 160.8 (CO), 136.0 (thiophene 2-C), 135.8 (isoquinoline 3-CH), 134.1 (isoquinoline 6-CH), 128.8 (isoquinoline 4a-C), 128.6 (isoquinoline 8a-C), 128.3 (thiophene 5-CH), 127.0 (isoquinoline 7-CH), 126.4 (isoquinoline 8-CH and 5-CH), 125.7 (4'-CH), 124.1 (4-C), 123.5 (3'-CH), 47.3 (CH_2); MS (FAB +ve) *m/z* 287.0488 (M+H) ($C_{14}H_{11}O_3N_2S$ requires 287.0490).



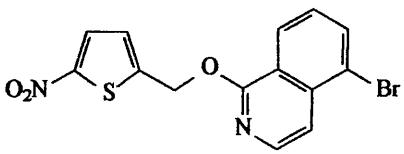
1-(5-nitro-2-thienylmethoxy)isoquinoline (139)

Diethyl azodicarboxylate (0.20 ml, 1.2 mmol) was added dropwise to isoquinolin-1-one **28** (0.09 g, 0.6 mmol) and PPh_3 (0.33 g, 1.2 mmol) in dry THF (20 ml) under dry Ar. The mixture was stirred for 15 min and **119** (0.1 g, 0.6 mmol) was added. The mixture was stirred overnight. Evaporation and chromatography (EtOAc/hexane 1:1) afforded **139** (67 mg, 38%) as a yellow oil: Rf 0.51 (EtOAc/hexane 1:1); IR 3061, 2970, 1735, 1631, 1439, 1398 cm^{-1}; ^1H NMR ($CDCl_3$) δ.8.25 (1 H, d, J = 8.2 Hz, isoquinoline 8-H), 8.00 (1 H, d, J = 6.0 Hz, isoquinoline 3-H), 7.8 (1 H, d, J = 4.0 Hz, thiophene 4-H), 7.75 (1 H, d, J = 7.7 Hz, isoquinoline 5-H), 7.7 (1 H, t, J = 7.7 Hz, isoquinoline 6-H), 7.55 (1 H, t, J = 7.7 Hz, isoquinoline 7-H), 7.3 (1 H, d, J = 6.0 Hz, isoquinoline 4-H), 7.12 (1 H, d, J = 4.0 Hz, thiophene 3-H), 5.74 (2 H, s, CH_2); ^{13}C NMR ($CDCl_3$) δ 165.6 (CO), 158.9 (thiophene 5-C), 148.2 (thiophene 2-C), 131.8 (isoquinoline 3-CH), 131.5 (isoquinoline 4a-C), 132.3 (isoquinoline 6-CH), 132.1 (thiophene 4-CH), 130.0 (isoquinoline 5-CH), 128.5 (isoquinoline 7-CH), 128.1 (isoquinoline 8a-C), 127.4 (isoquinoline 8-CH), 126.1 (thiophene 3-CH), 116.1 (isoquinoline 4-CH), 62.3 (CH_2); MS (FAB +ve) *m/z* 287.0486 (M+H) ($C_{14}H_{11}N_2O_3S$ requires 287.0490).



5-Iodo-1-(5-nitro-2-thienylmethoxy)isoquinoline (140)

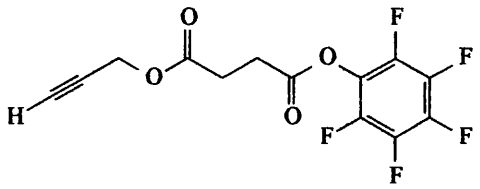
Diethyl azodicarboxylate (0.12 ml, 0.7 mmol) was added dropwise to 5-iodoisoquinolin-1-one **34** (0.1 g, 0.4 mmol) and PPh_3 (0.19 g, 0.7 mmol) in dry THF (20 ml) under dry Ar. The mixture was stirred for 15 min and **119** (0.06 g, 0.4 mmol) was added. The mixture was stirred overnight. Evaporation and chromatography (EtOAc/hexane 1:1) afforded **140** (45 mg, 30%) as a yellow powder: Rf 0.54 (EtOAc/hexane 1:1); mp 78-82°C; IR 3066, 2968, 1618, 1459, 1387 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.27 (1 H, d, J = 8.5 Hz, isoquinoline 8-H), 8.22 (1 H, d, J = 7.4 Hz, isoquinoline 6-H), 8.10 (1 H, d, J = 6.2 Hz, isoquinoline 3-H), 7.83 (1 H, d, J = 3.9 Hz, thiophene 4-H), 7.49 (1 H, d, J = 6.2 Hz, isoquinoline 4-H), 7.27 (1 H, t, J = 8.2 Hz, isoquinoline 7-H), 7.12 (1 H, d, J = 3.9 Hz, thiophene 3-H), 5.74 (2 H, s, CH_2); ^{13}C NMR ($CDCl_3$) δ 158.9 (isoquinoline 1-C), 147.6 (thiophene 5-C), 141.7 (isoquinoline 4a-C and 6-CH), 140.5 (thiophene 2-C), 139.6 (isoquinoline 3-CH), 127.9 (isoquinoline 7-CH and 8-CH), 125.9 (thiophene 4-CH), 124.3 (isoquinoline 8a-C), 120.0 (thiophene 3-CH), 119.7 (isoquinoline 4-CH), 96.9 (isoquinoline 5-C), 62.8 (CH_2); MS found (EI) *m/z* 411.9360 (M) ($C_{14}H_9IN_2O_3S$ requires 411.9378). Anal. C 40.70%, H 2.09%, N 6.43% ($C_{14}H_9IN_2O_3S$ requires C 40.87%, H 2.18%, N 6.80%).



5-Bromo-1-(5-nitro-2-thienylmethoxy)isoquinoline (141)

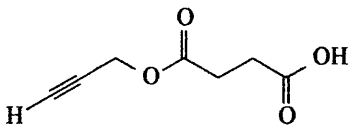
Diethyl azodicarboxylate (0.2 ml, 1.2 mmol) was added dropwise to 5-bromoisoquinolin-1-one **35** (0.14 g, 0.6 mmol) and PPh_3 (0.33 g, 1.2 mmol) in dry THF (20 ml) under dry Ar. The mixture was stirred for 15 min and **119** (0.1 g, 0.6 mmol) was added. The mixture was stirred overnight. Evaporation and chromatography (EtOAc/hexane 1:1) afforded **141** (94 mg, 41%) as a yellow powder: Rf 0.5 (EtOAc/hexane

1:1); mp 128-130°C; IR 3098, 2968, 1616, 1459,1399 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.25 (1 H, d, J = 8.2 Hz, isoquinoline 8-H), 8.12 (1 H, d, J = 6.0 Hz, isoquinoline 3-H), 7.97 (1 H, d, J = 7.8 Hz, isoquinoline 6-H), 7.84 (1 H, d, J = 4.2 Hz, thiophene 4-H), 7.65 (1 H, d, J = 6.0 Hz, isoquinoline 4-H), 7.42 (1 H, t, J = 7.7 Hz, isoquinoline 7-H), 7.13 (1 H, d, J = 4.2 Hz, thiophene 3-H), 5.75 (2 H, s, CH_2); ^{13}C NMR ($CDCl_3$) δ 158.9 (isoquinoline 1-C), 147.8 (thiophene 5-C), 140.6 (isoquinoline 3-CH), 139.0 (thiophene 2'-C and isoquinoline 4a-C), 134.7 (isoquinoline 6-CH), 128.2 (thiophene 4-CH), 127.5 (isoquinoline 7-CH), 126.1 (isoquinoline 8a-C), 123.7 (isoquinoline 8-CH), 121.5 (thiophene 3-CH), 120.1 (isoquinoline 4-CH), 115.2 (isoquinoline 5-C), 62.9 (CH_2); MS (FAB +ve) *m/z* 366.9575 (M+H) ($C_{14}{}^{81}BrH_{10}N_2O_3S$ requires 366.9589), 365 (M+H for $C_{14}{}^{79}BrH_{10}N_2O_3S$).



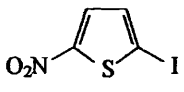
Pentafluorophenyl prop-2-ynyl butanedioate (146)

Compound **147** (0.50 g, 3.2 mmol) in EtOAc (10 ml) was stirred at 0°C for 15 min. Pentafluorophenol (0.59 g, 3.2 mmol) in EtOAc (5 ml) was added to the solution followed by 1,3-dicyclohexylcarbodiimide (0.66 g, 3.2 mmol) in EtOAc (5 ml). The mixture was stirred for 5 h and was allowed to stand at 4°C overnight. Filtration and evaporation gave **146** (1.12 g, 100%) as a light yellow oil: Rf 0.6 (EtOAc/hexane 1:1); IR 3250, 2960, 1760-1720, 1500, 1000 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.74 (2 H, s, CH_2O), 3.03 (2 H, t, J = 6.6 Hz, $COCH_2$), 2.80 (2 H, t, J = 6.6 Hz, CH_2CO), 2.50 (1 H, s, C≡CH); ^{13}C NMR ($CDCl_3$) δ 170.8 (1-CO), 167.9 (4-CO), 76.8 (2'-C), 75 (1'-CH), 52.1 (CH_2), 28.5 (2 and 3-CH_2); owing to multiple C-F coupling and to the quaternary nature of these carbons, the signals for the C_6F_5 carbons were too weak to be observed; MS (FAB +ve) *m/z* 323.0347 (M+H) ($C_{13}H_8F_5O_4$ requires 323.0342).



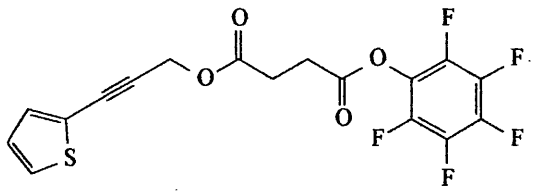
4-Oxo-4-(prop-2-ynyloxy)butanoic acid (147)

Succinic anhydride **125** (1.78 g, 18 mmol) was stirred with pyridine (15 ml, 185 mmol), DMAP (5 mg) and propargyl alcohol **148** (1.0 g, 18 mmol) overnight. The evaporation residue in EtOAc was washed with water and dried. Evaporation yielded **147** (1.87 g, 67%) as a light yellow oil: Rf 0.1 (EtOAc/hexane 1:1); IR, 3500, 3250, 2960, 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.35 (1 H, s, OH), 4.71 (2 H, s, CH_2O), 2.88 (4 H, m, CH_2CH_2), 2.49 (1 H, s, CH); ^{13}C NMR ($CDCl_3$) δ 177.5 (1-CO), 171.7 (4-CO), 76.7 (*C*≡CH), 75.0 (C≡*C*H), 52.6 (CH_2), 29.2 (CH_2CH_2); MS (FAB +ve) *m/z* 157.0511 (M+H) ($C_7H_9O_4$ requires 156.0500).



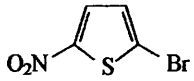
2-Iodo-5-nitrothiophene (150)

60% nitric acid (0.42 ml, 9.5 mmol) was added to acetic anhydride (2.0 ml, 0.21 mmol) at –5°C. The mixture was cooled to -10°C and 2-iodothiophene **149** (1.0 g, 4.7 mmol) in acetic anhydride was added dropwise. The mixture was stirred at -10 °C for 2 h and poured into an ice water mixture. The precipitate was recrystallised (MeOH) to yield **150** (0.9 g, 60%) as buff shiny crystals: Rf 0.5 (EtOAc/hexane 1:1); mp 60-61°C (lit.[162] mp 70-75°C); ^1H NMR ($CDCl_3$) δ 7.55 (1 H, d, J = 4.3 Hz, 4-H), 7.30 (1 H, d, J = 4.3 Hz, 3-H).



1-Pentafluorophenyl-4-[3-(2-thienyl)prop-2-ynyl] butanedioate (151)

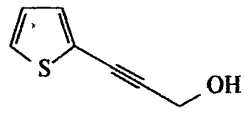
Et_3N (0.59 ml, 4.3 mmol) was added to **146** (0.2 g, 0.6 mmol), benzene (5 ml), 2-iodothiophene (0.09 ml, 0.4 mmol), $Pd(PPh_3)_4$ (20 mg) and CuI (10 mg) under dry Ar. The mixture was stirred for 24 h. MeOH (5 ml) was added, the solvent was evaporated and diethyl ether (20 ml) was added to the residue leading to the precipitation of Et_3NH^+ I⁻. This mixture was filtered, washed with 10% aq. HCl, water, brine and dried. Evaporation and chromatography (hexane/EtOAc 1:1) yielded **151** (0.09 g, 52%) as a yellow oil: Rf 0.7 (EtOAc/hexane 1:1); IR (KBr) 3091, 2962, 1669, 1059, 1404, 1326, 1262 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.28 (1 H, dd, J = 5.4, 1.1 Hz, thiophene 5-H), 7.24 (1 H, dd, J = 3.5, 1.2 Hz, thiophene 3-H), 6.97 (1 H, dd, J = 5.4, 3.5 Hz, thiophene 4-H), 4.97 (2 H, s, CH_2O), 3.03 (2 H, t, J = 6.6 Hz, CH_2CO), 2.85 (2 H, t, J = 6.6 Hz, $COCH_2$); ^{13}C NMR ($CDCl_3$) δ 170.0 (CO), 167.9 (CO), 132.9 (thiophene 3-CH), 128.5 (thiophene 4-CH), 127.5 (thiophene 5-CH), 121.7 (thiophene 2-C), 86.4 ($C{\equiv}CCH_2$), 80.1 ($C{\equiv}CCH_2$), 53.4 ($C{\equiv}CCH_2$), 28.4 (3 and 2-CH_2); owing to multiple C-F coupling and to the quaternary nature of these carbons, the signals for the C_6F_5 carbons were too weak to be observed; MS (FAB +ve) *m/z* 405.0214 (M+H) ($C_{17}H_{10}F_5O_4$ requires 405.0219).



2-Bromo-5-nitrothiophene (152)

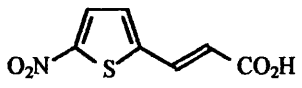
HNO_3 (15.5 M) (5.06 ml, 79 mmol) in Ac_2O at 0°C was added dropwise to 2-bromo-thiophene (5.0 g, 0.36 mmol) in Ac_2O (10 ml) at -5°C during 45 min. Stirring was continued for another 30 min. The mixture was kept at 4°C overnight and was poured over crushed ice with stirring. Chromatography (EtOAc) yielded **152** (3.8 mg, 60%)

as a shiny buff crystalline solid: Rf 0.61 (EtOAc); mp 42-44°C (lit.[178] mp 45-46°C); ^{1}H NMR ($CDCl_3$) δ 7.70 (1 H, d, J = 4.3 Hz, 4-H), 7.12 (1 H, d, J = 4.3 Hz, 3-H).



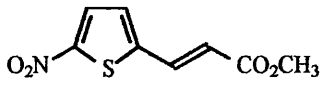
3-(2-Thienyl)prop-2-yn-1-ol (153)

Et_3N (6.6 ml, 47 mmol) was added to propargyl alcohol **148** (0.41 ml, 7.1 mmol), benzene (50 ml), 2-iodothiophene **149** (0.6 ml, 4.7 mmol), $Pd(PPh_3)_4$ (200 mg) and CuI (100 mg) under dry Ar. The mixture was stirred for 24 h. MeOH (50 ml) was added, the solvent was evaporated and diethyl ether (20 ml) was added to the residue leading to the precipitation of Et_3NH^+ I^-. This mixture was filtered off, washed with 10% aq. HCl, water, brine and dried. Evaporation and chromatography (hexane/ EtOAc 1:1) yielded **153** (0.43 g, 67%) as a yellow oil: Rf 0.4 (EtOAc/hexane 1:1); ^{1}H NMR ($CDCl_3$) δ 7.24 (1 H, d, J = 5.0 Hz, thiophene 5-H), 7.20 (1 H, d, J = 3.5 Hz, thiophene 3-H), 6.94 (1 H, dd, J = 3.5, 5.0 Hz, thiophene 4-H), 4.48 (2 H, s, CH_2); ^{13}C NMR ($CDCl_3$) δ 132.1 (thiophene 3-CH), 127.2 (thiophene 4-CH), 126.1 (thiophene 5-CH), 122.2 (thiophene 2-C), 91.2 ($C{\equiv}CCH_2$), 78.7 ($C{\equiv}CCH_2$), 51.4 ($C{\equiv}CCH_2$).



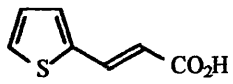
3-(5-Nitro-2-thienyl)propenoic acid (157)

5-Nitrothiophene-2-carboxaldehyde **118** (1.0 g, 6.4 mmol), propanedioic acid (1.8 g, 17 mmol), pyridine (5 ml) and piperidine (1 ml) were heated at 100°C for 2 h and then boiled for 5 min. After cooling, the solution was poured into water and was treated with excess aq. HCl. The precipitate was filtered and recrystallised (aq. MeOH) to yield **157** (0.85 g, 85%) as a light buff solid: mp >230°C (lit.[173] mp 255-256°C); ^{1}H NMR ($CDCl_3$) δ 12.50 (1 H, s, OH), 7.90 (1 H, d, J = 4.4 Hz, thiophene 4-H), 7.65 (1 H, d, J = 15.7 Hz, 3-H), 7.40 (1 H, d, J = 4.4 Hz, thiophene 3-H), 6.40 (1 H, d, J = 15.7 Hz, 2-H).



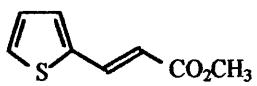
Methyl 3-(5-nitro-2-thienyl)-3-propenoate (158)

A suspension of **157** (0.10 g, 0.5 mmol) in MeOH (3.3 ml) and conc. H_2SO_4 (0.66 ml) was stirred under reflux overnight. The mixture was cooled to 20°C and water was added (7 ml). The precipitate was filtered, washed with aq. $NaHCO_3$ and recrystallised (acetone/water) to yield **158** (0.094 g, 88%) as a light buff crystalline solid: mp 153-155°C (lit.[174] mp 157-158°C); ^{1}H NMR (DMSO) δ 8.05 (1 H, d, J = 4.4 Hz, thiophene 4-H), 7.80 (1 H, d, J = 16.0 Hz, 3-H), 7.63 (1 H, d, J = 4.4 Hz, thiophene 3-H), 6.70 (1 H, d, J = 16.0 Hz, 2-H), 3.50 (3 H, s, CH_3O).



2-Thienyl-3-propenoic acid (160)

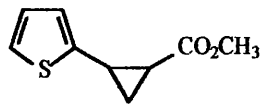
Thiophene-2-carboxaldehyde **130** (1.0 g, 8.9 mmol), propanedioic acid (1.8 g, 12 mmol) pyridine (5 ml) and piperidine (1 ml) were heated at 100°C for 2 h and then boiled for 5 min. After cooling, the solution was poured into water and treated with excess aq. HCl. The precipitate was filtered and recrystallised (aq. MeOH) to yield **160** (0.97 g, 70%) as a light buff solid: mp 142-143°C (lit.[163] mp 143-144°C); ^{1}H NMR ($CDCl_3$) δ 12.50 (1 H, s, OH), 7.88 (1 H, d, J = 15.5 Hz, 3-H), 7.41 (1 H, d, J = 5.1 Hz, thiophene 5-H), 7.30 (1 H, d, J = 3.3 Hz, thiophene 3-H), 7.08 (1 H, dd, J = 5.1, 3.3 Hz, thiophene 4-H), 6.24 (1 H, d, J = 15.5 Hz, 2-H).



Methyl 2-thienyl-3-propenoate (161)

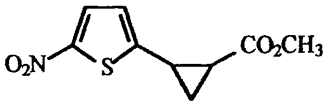
Compound **160** (0.10 g, 0.5 mmol) in MeOH (3.3 ml) and conc. H_2SO_4 (0.66 ml) was stirred under reflux overnight. The mixture was then cooled to 20°C and water (7 ml) was added. The precipitate was filtered, washed with aq. $NaHCO_3$ and recrystallised

(acetone/water) to yield **161** (0.86 g, 83%) as a shiny off-white crystalline solid: mp 54-55°C (lit.[175] mp 52-54°C); ^{1}H NMR ($CDCl_3$) δ 7.79 (1 H, d, J = 15.5 Hz, 3-H), 7.37 (1 H, d, J = 5.1 Hz, thiophene 5-H), 7.25 (1 H, d, J = 3.3 Hz, thiophene 3-H), 7.05 (1 H, dd, J = 5.0, 3.3 Hz, thiophene 4-H), 6.25 (1 H, d, J = 15.5 Hz, 2-H), 3.78 (3 H, s, CH_3).



2-(2-Thienyl)cyclopropane-1-carboxylate (162)

Diazomethane was prepared as previously described, a solution of N-methyl-N-nitroso-4-toluenesulfonamide (Diazald® 2.5g, 11.67 mmol) in diethyl ether (20 ml) was slowly added to a heated (65°C on an oil bath) mixture of KOH (2.5 g, mmol), water (4 ml), and ethanol (5 ml). the resulting ether solution of diazomethane was distilled and transferred by cannula into a flask containing **161** (77 mg, 0.36 mmol), $Pd(OAc)_2$ (23 mg, 1.0 mmol) in diethyl ether (5 ml). The mixture was then stirred for 1 h. The reaction was quenched by the addition of acetic acid (0.5 ml). The evaporation residue in diethyl ether was washed with aq. $NaHCO_3$ and dried. Evaporation and chromatography (EtOAc/hexane 1:1) gave **162** (52 mg, 80%) as a brown oil: Rf 0.57 (EtOAc/hexane 1:1); IR 3001, 2951, 1727, 1628, 1438, 1205, 1172 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.09 (1 H, dd, J = 5.0, 1.5 Hz, thiophene 5-H), 6.89 (1 H, dd, J = 5.5, 3.5 Hz, thiophene 4-H), 6.81 (1 H, d, J = 3.5 Hz, thiophene 3-H), 3.7 (3 H, s, CH_3), 2.7 (1 H, m, 2-CH), 1.9 (1 H, m, 1-CH), 1.6 (1 H, m) and 1.3 (1 H, m) (3-H_2); ^{13}C NMR ($CDCl_3$) δ 173.3 (CO), 144.1 (thiophene 2-C), 127.1 (thiophene 5-CH), 124.2 (thiophene 3-CH), 123.4 (thiophene 4-CH), 52.3 (CH_3), 25.1 (2-CH), 21.9 (1-CH), 18.2 (3-CH_2); MS (FAB +ve) *m/z* 183 (M+H).



Methyl 2-(5-nitro-2-thienyl)cyclopropanecarboxylate (163)

Conc. HNO_3 (0.05 ml, 0.8 mmol) was added to compound **162** (40 mg, 0.2 mmol) in TFA (2 ml) at –10°C and the mixture was stirred for 1 h at –10°C. The pH of the mixture was adjusted to 5 with 2 M aq. NaOH and the mixture was extracted with EtOAc. The organic layer was washed with water, brine and dried. Evaporation and chromatography (EtOAc/hexane 1:1) yielded **163** (0.04 mg, 80%) as a dark yellow oil: Rf 0.41 (EtOAc/hexane 1:1); IR 3006, 2953, 1728, 1647, 1537, 1336, 1438, 1396, 1207, 1174 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.75 (1 H, d, J = 4.3 Hz, thiophene 4-H), 6.79 (1 H, d, J = 4.3 Hz, thiophene 3-H), 3.8 (3 H, s, CH_3), 2.65 (1 H, m, 2-H), 1.76 (1 H, m, 1-H), 1.4 (1 H, m) and 1.3 (1H, m) (3-H_2); ^{13}C NMR ($CDCl_3$) δ 172.8 (CO), 152.9 (thiophene 5-C), 144.5 (thiophene 2-C), 128.7 (thiophene 4-CH), 125.6 (thiophene 3-CH), 52.2 (CH_3O), 21.0 (2-CH), 18.4 (1-CH), 14.2 (3-CH_2); MS (EI) *m/z* 227.0244 (M), ($C_9H_9NO_4S$ requires 227.0252).

Release Studies

Procedure 1

Previously dried **39/40/65/94/96/97/99** (10 mg) were dissolved in $CDCl_3$ (0.6 ml) and transferred in an NMR tube. The ^1H NMR spectrum of each sample was run on the Varian EX400 spectrometer, followed by the gradual addition of $SnCl_2$ in deuterated methanol by portions of 0.1 eq. A proton NMR analysis was run after each addition.

Procedure 2

Previously dried **65** (10 mg) preliminary dried was dissolved in in $CDCl_3$ (0.6 ml) and transferred in an NMR tube. The ^1H NMR of the sample was run on the Varian EX400 spectrometer, followed by the gradual addition of $Na_2S_2O_5$ in deuterated methanol by portions of 0.1 eq. A proton NMR analysis was run after each addition.

Procedure 3

$SnCl_2$ (0.5 g) was added to **42/97/129/139/140/141** (0.5 mg) in methanol (2 ml). The mixture was stirred. Aliquots (100 µl) were removed at regular time points and analysed by HPLC.

Procedure 4

$SnCl_2$ (0.5 g) was added to **129** (0.5 mg) in methanol (2 ml). The mixture was boiled under reflux. Aliquots (100 µl) were removed at regular time points and analysed by HPLC.

Procedure 5

Zn dust (0.5 mg) was added to **42** (0.5 mg) and NH_4Cl (0.5 mg) in MeOH (2 ml) and H_2O (0.05 ml). The mixture was stirred. Aliquots (100 µl) were removed at regular time points, filtered and analysed by HPLC.

Procedure 6

$NaBH_4$ (2.0 mg) was added to a mixture of **42/129/139/140/141** (5.0 mg), palladium on carbon (10%, 5.0 mg), and H_2O (0.04 ml) in isopropanol (5 ml). The mixture was stirred overnight. The evaporation residue was dissolved in deuterated methanol (0.6 ml) and submitted to proton NMR analysis.

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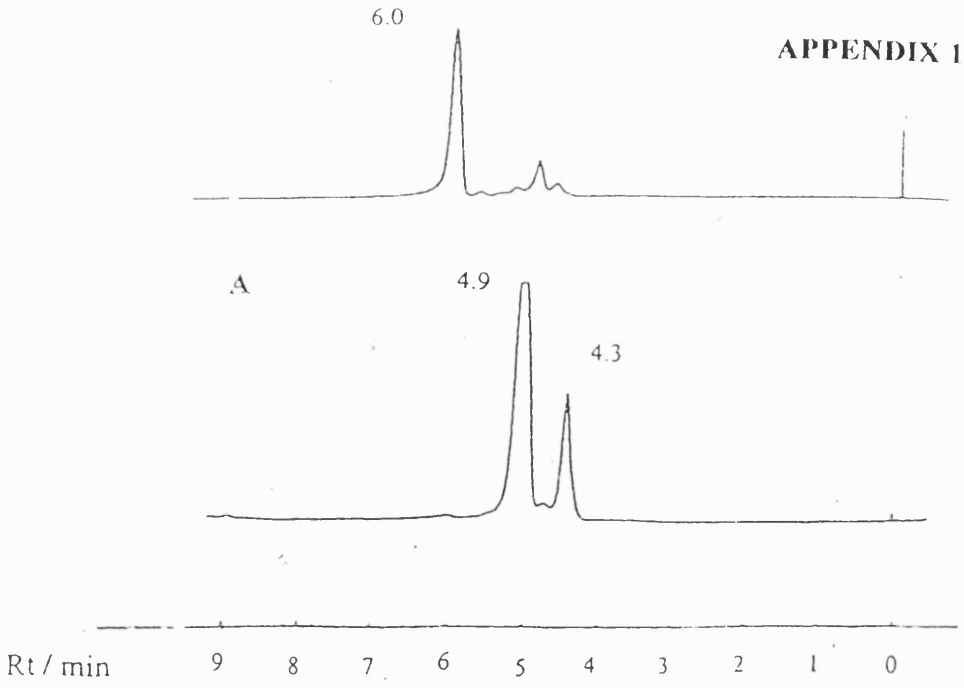
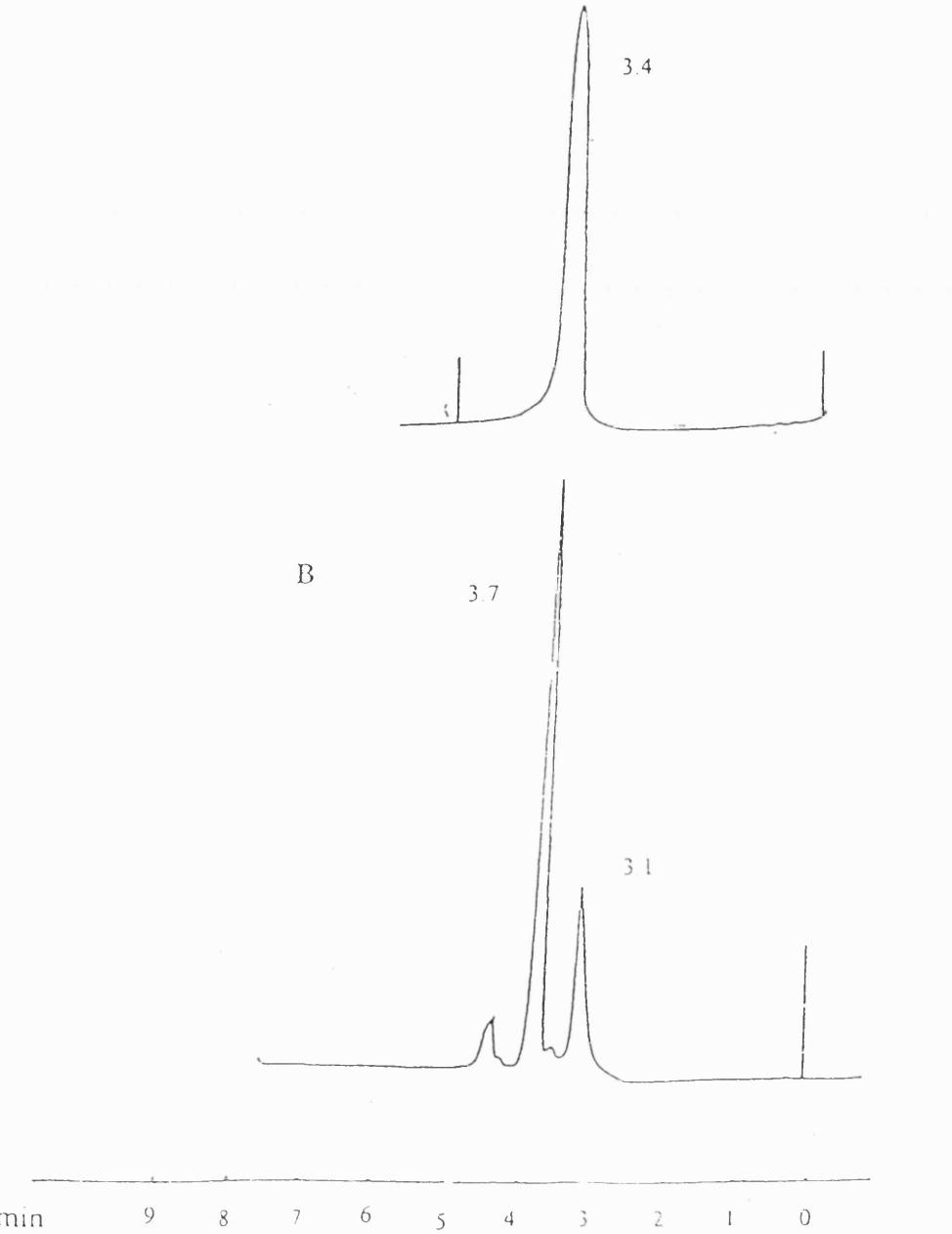
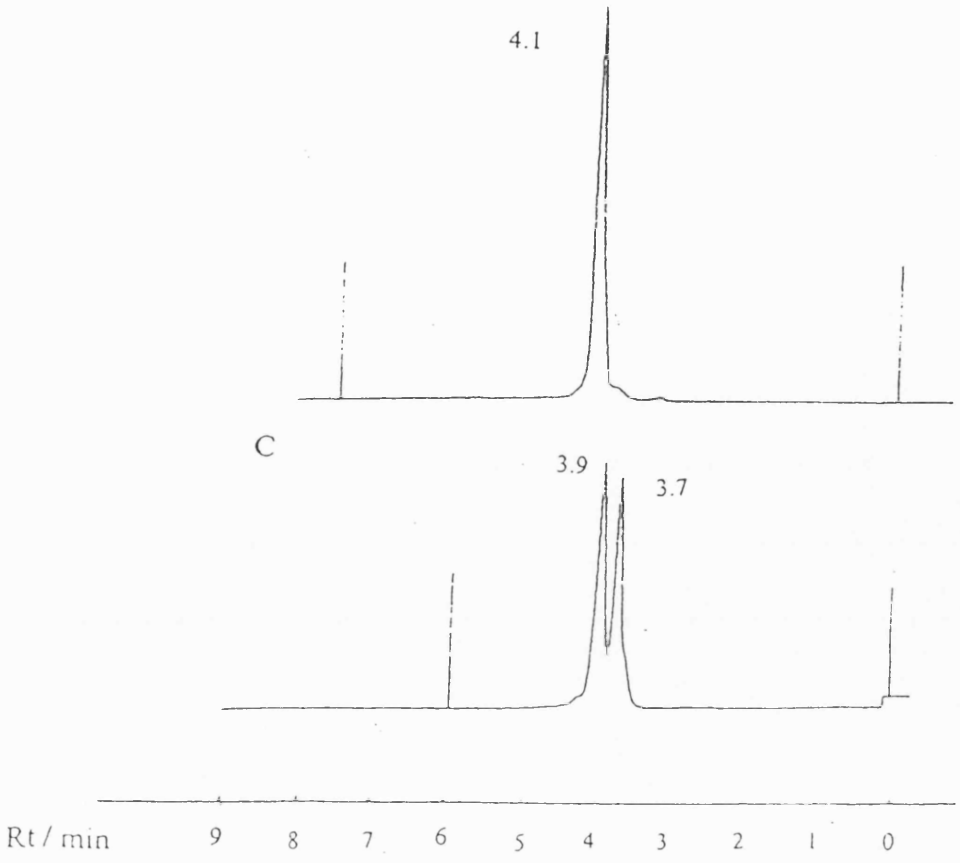
APPENDIX 1







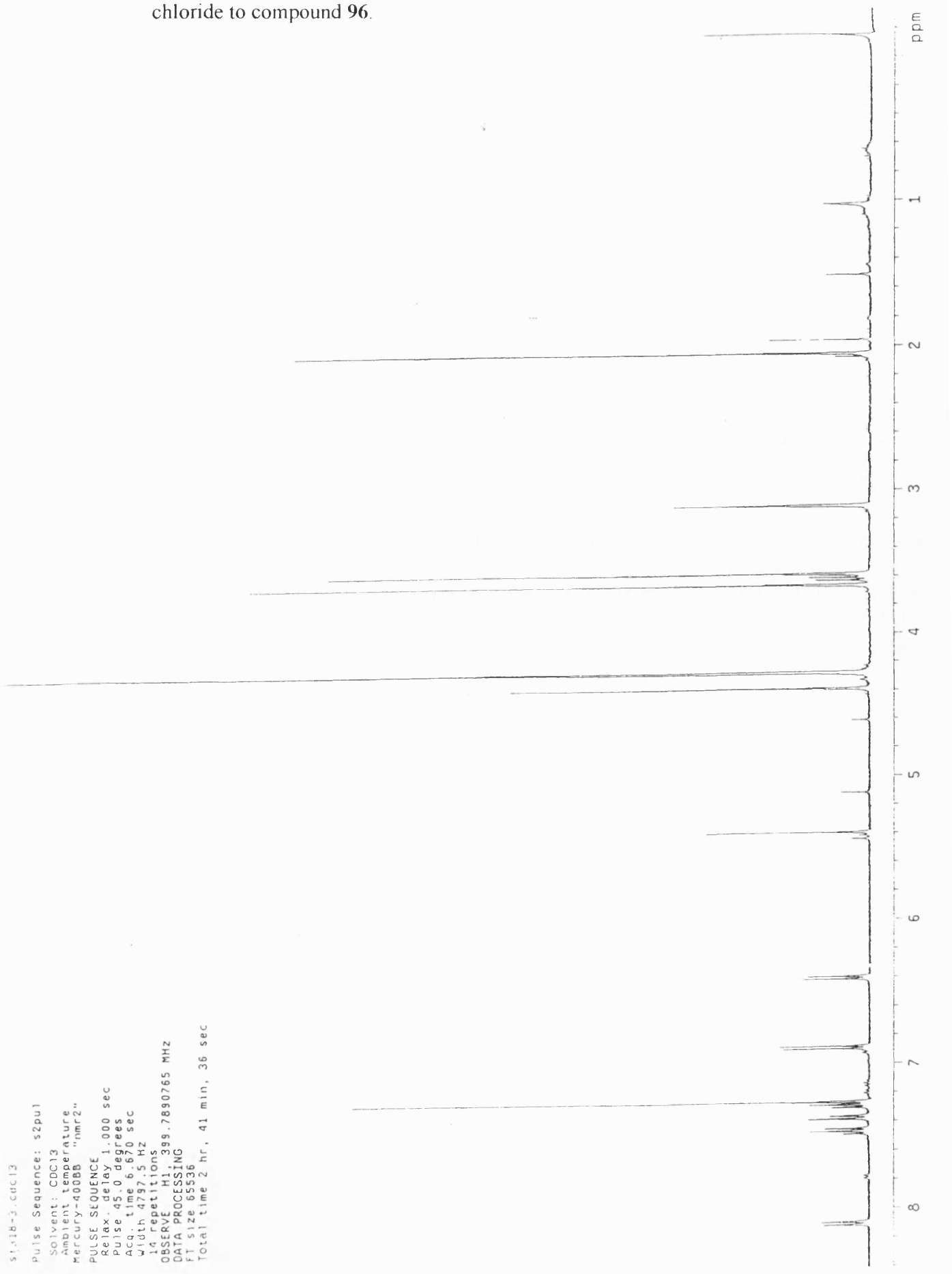
A, HPLC trace of compound **97** (top) and of the mixture obtained after addition of tin(II) chloride (bottom); **B**, HPLC trace of compound **42** (top) and of the mixture obtained after addition of zinc powder and ammonium chloride (bottom); **C**, HPLC trace of compound **129** (top) and of the mixture obtained after addition of tin(II) chloride and heating (bottom).

APPENDIX 2

^{1}H NMR spectrum of compound **96**.

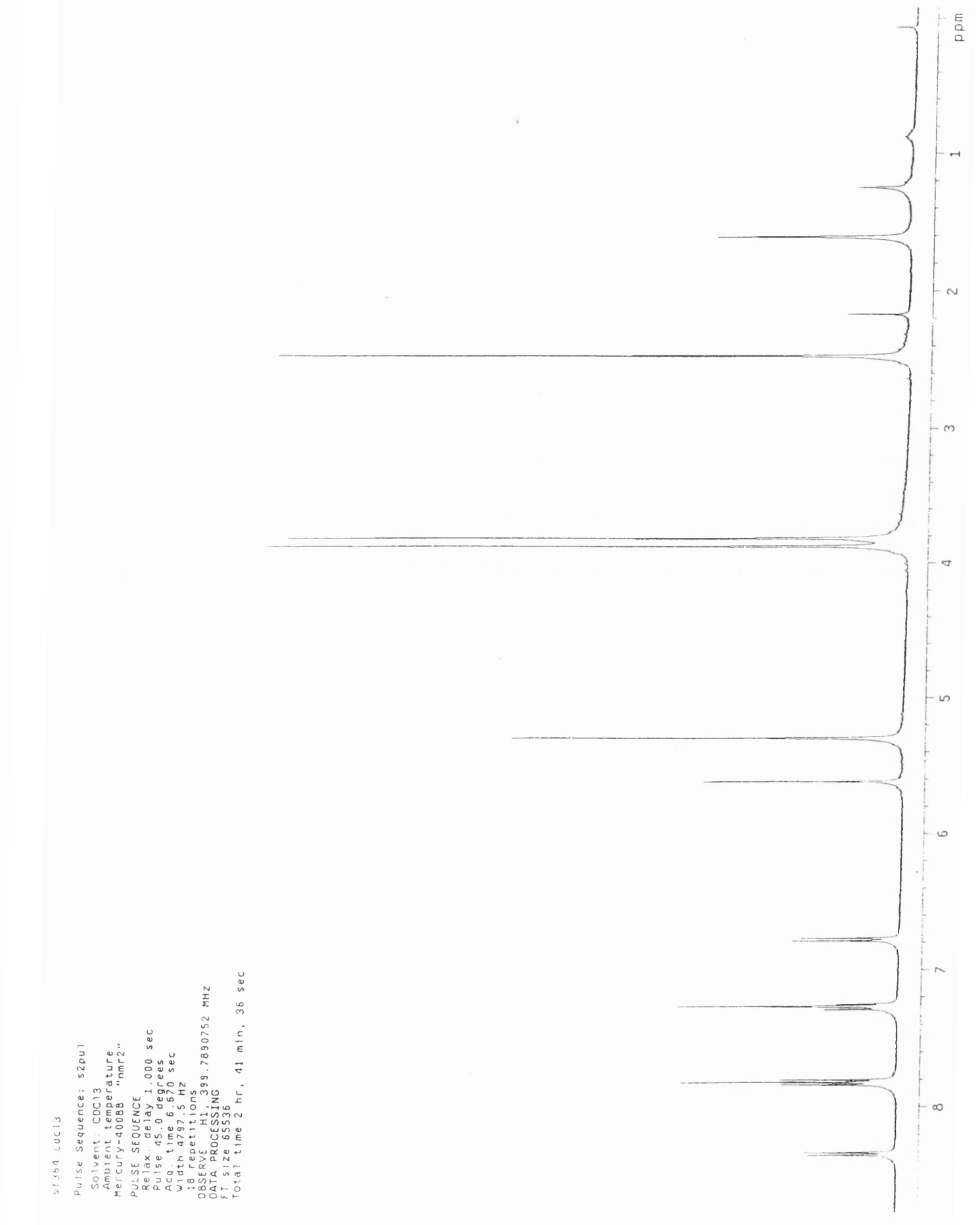
^{1}H NMR spectrum of the mixture obtained after addition of 0.5 equivalent of tin(II) chloride to compound **96**.

^{1}H NMR spectrum of the mixture obtained after addition of 1 equivalent of tin(II) chloride to compound **96**.

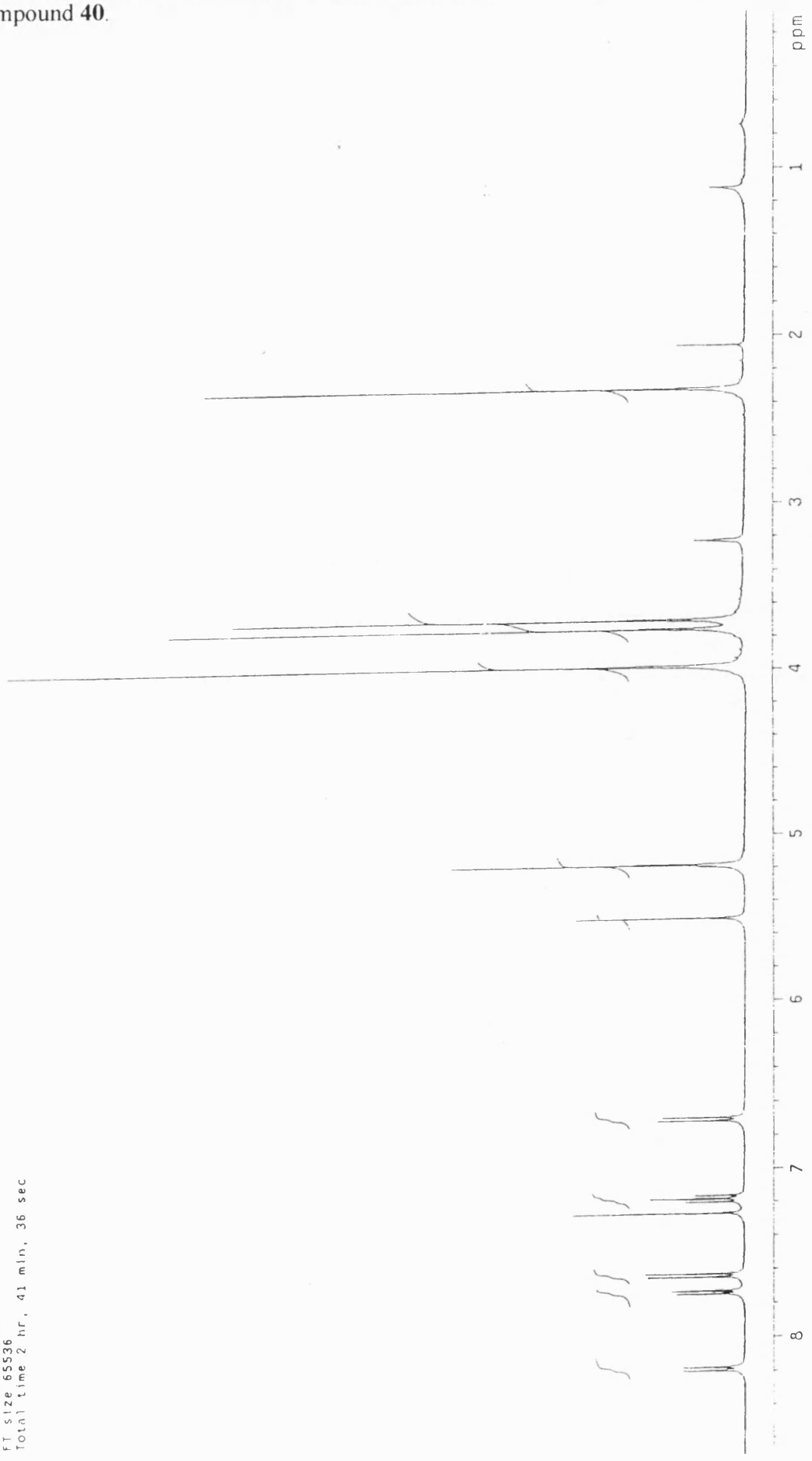


APPENDIX 5

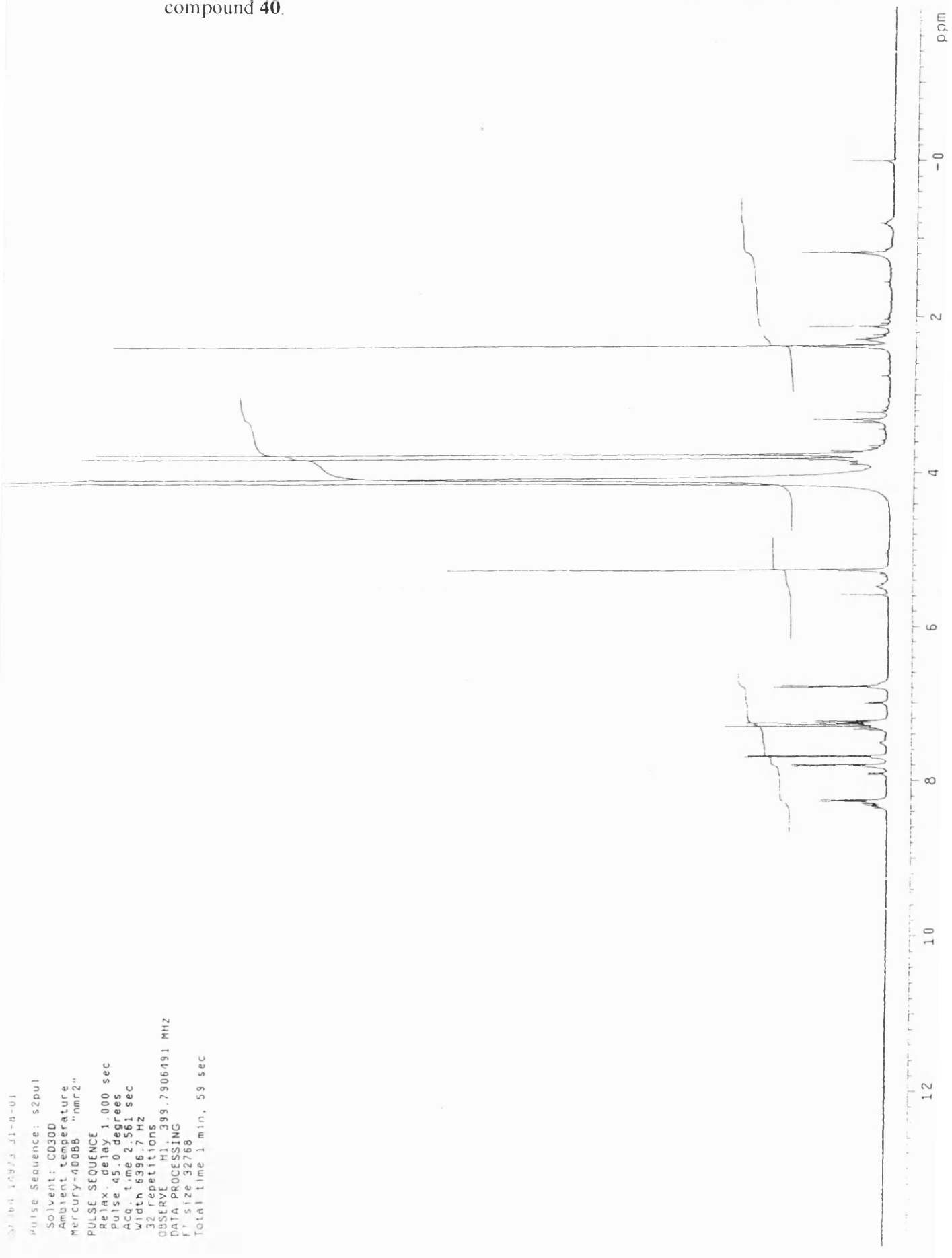
^{1}H NMR spectrum of compound **40**.



^{1}H NMR spectrum of the mixture obtained after addition of tin(II) chloride to compound **40**.



^{1}H NMR spectrum of the mixture obtained 24 h after addition of tin(II) chloride to compound **40**.



APPENDIX 8

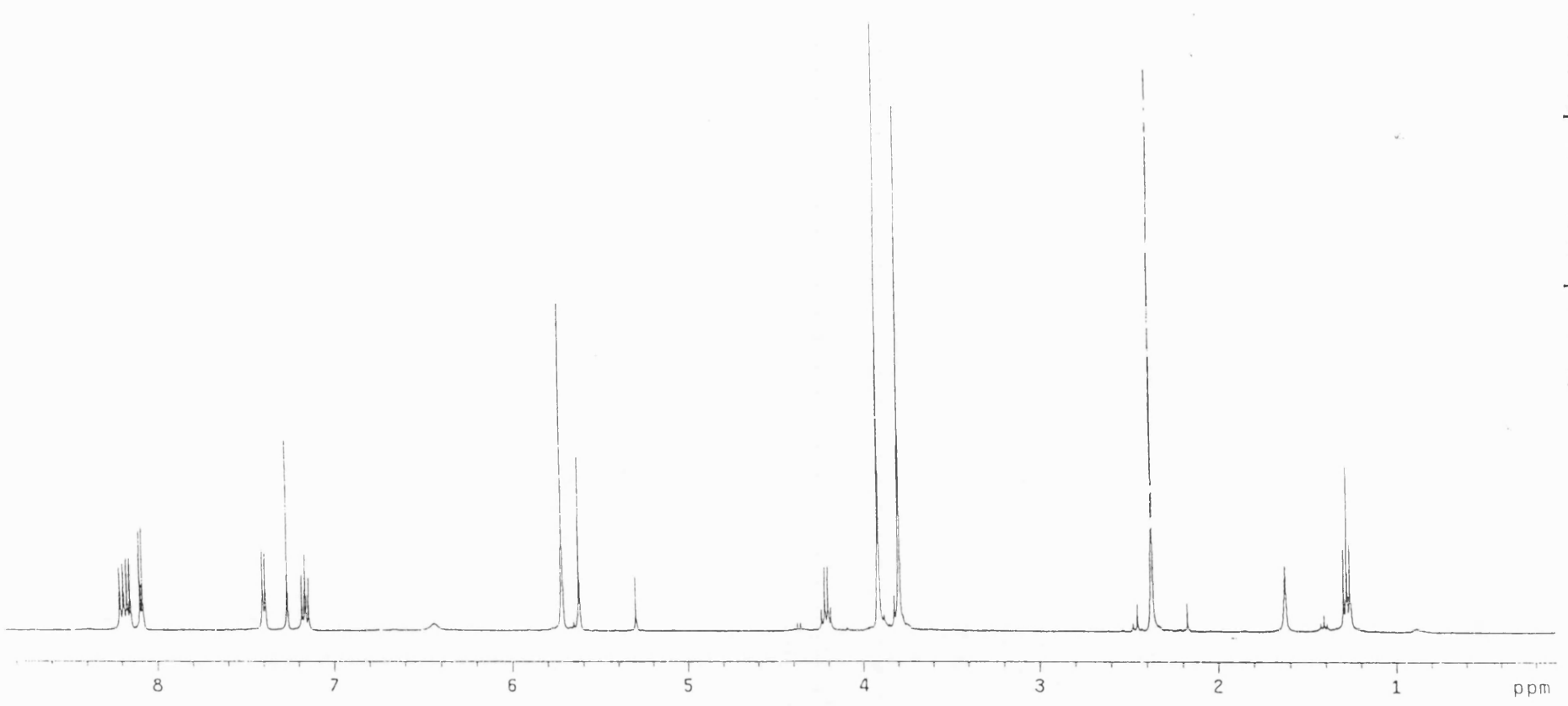
^{1}H NMR spectrum of the mixture obtained after addition of tin(II) chloride to compound **39**.

sf402.cdcl3

Pulse Sequence: s2pul

Solvent: CDCl3
Ambient temperature
Mercury-400BB "nmr2"

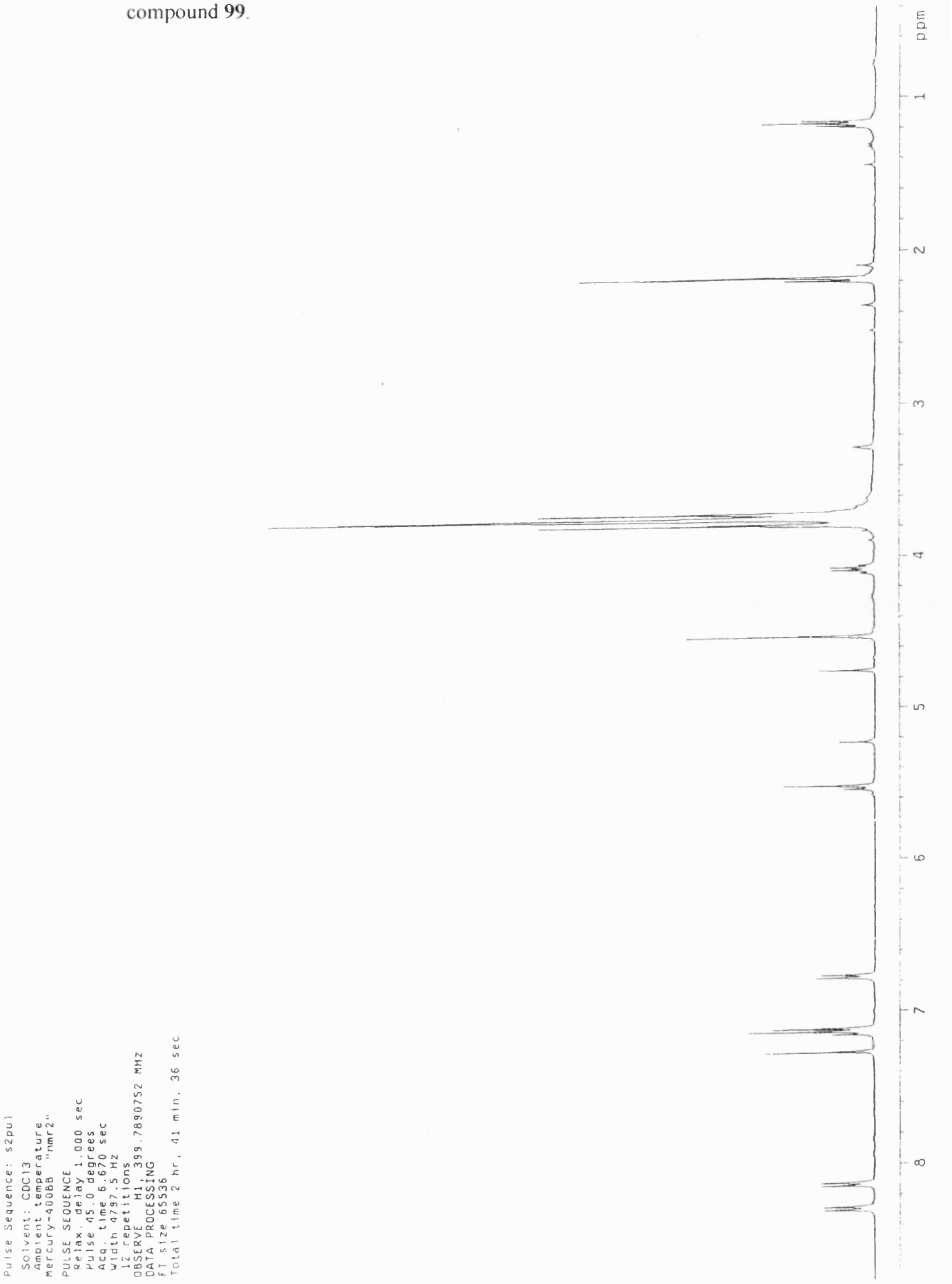
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 6.670 sec
Width 4797.5 Hz
32 repetitions
OBSERVE H1, 399.7890752 MHz
DATA PROCESSING
FT size 65536
Total time 2 hr, 41 min, 36 sec



^{1}H NMR spectrum of compound **99**.

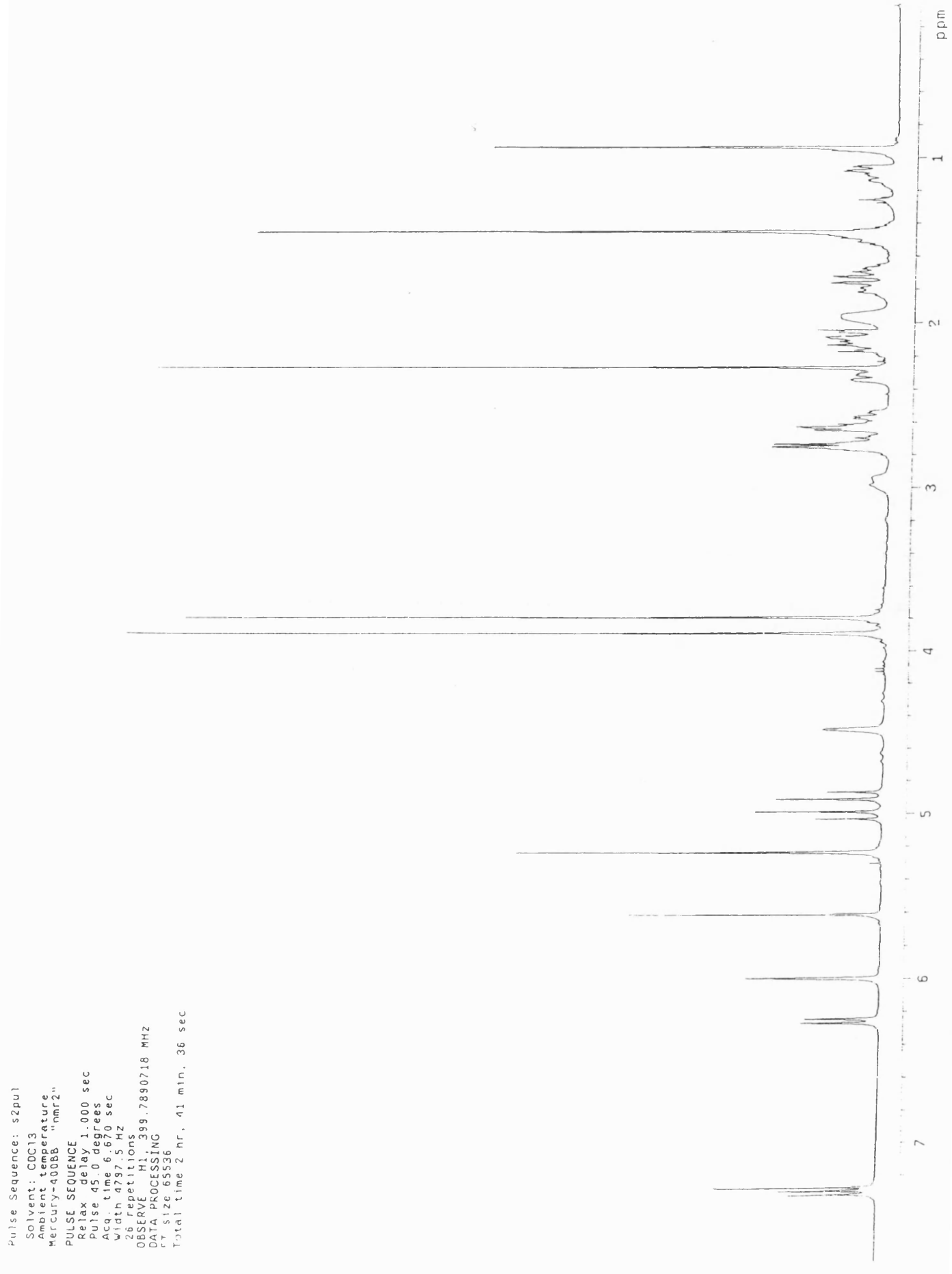
APPENDIX 9

^{1}H NMR spectrum of the mixture obtained after addition of tin(II) chloride to compound **99**.

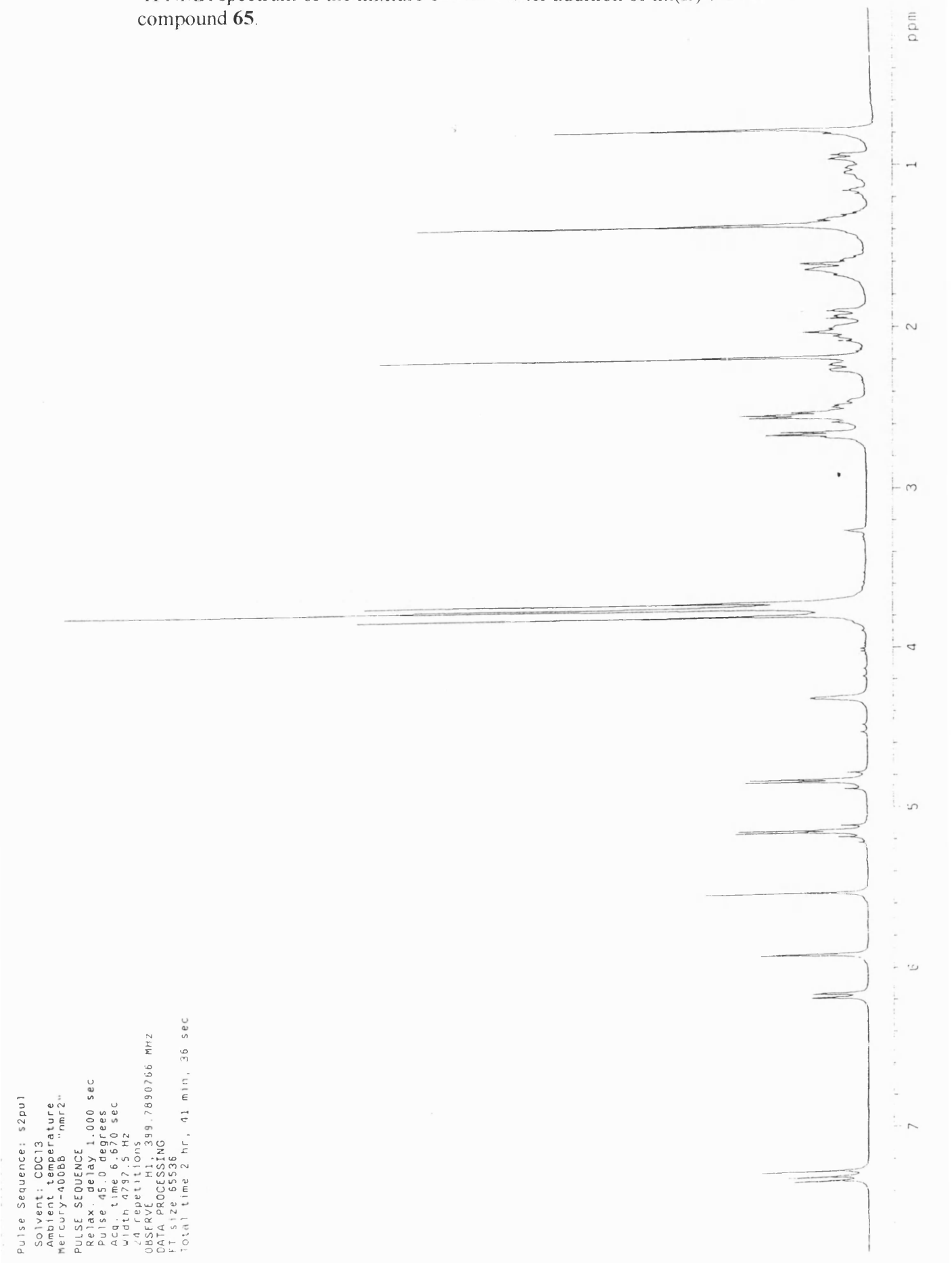


APPENDIX 11

^{1}H NMR spectrum of compound **65**.



^{1}H NMR spectrum of the mixture obtained after addition of tin(II) chloride to compound **65**.



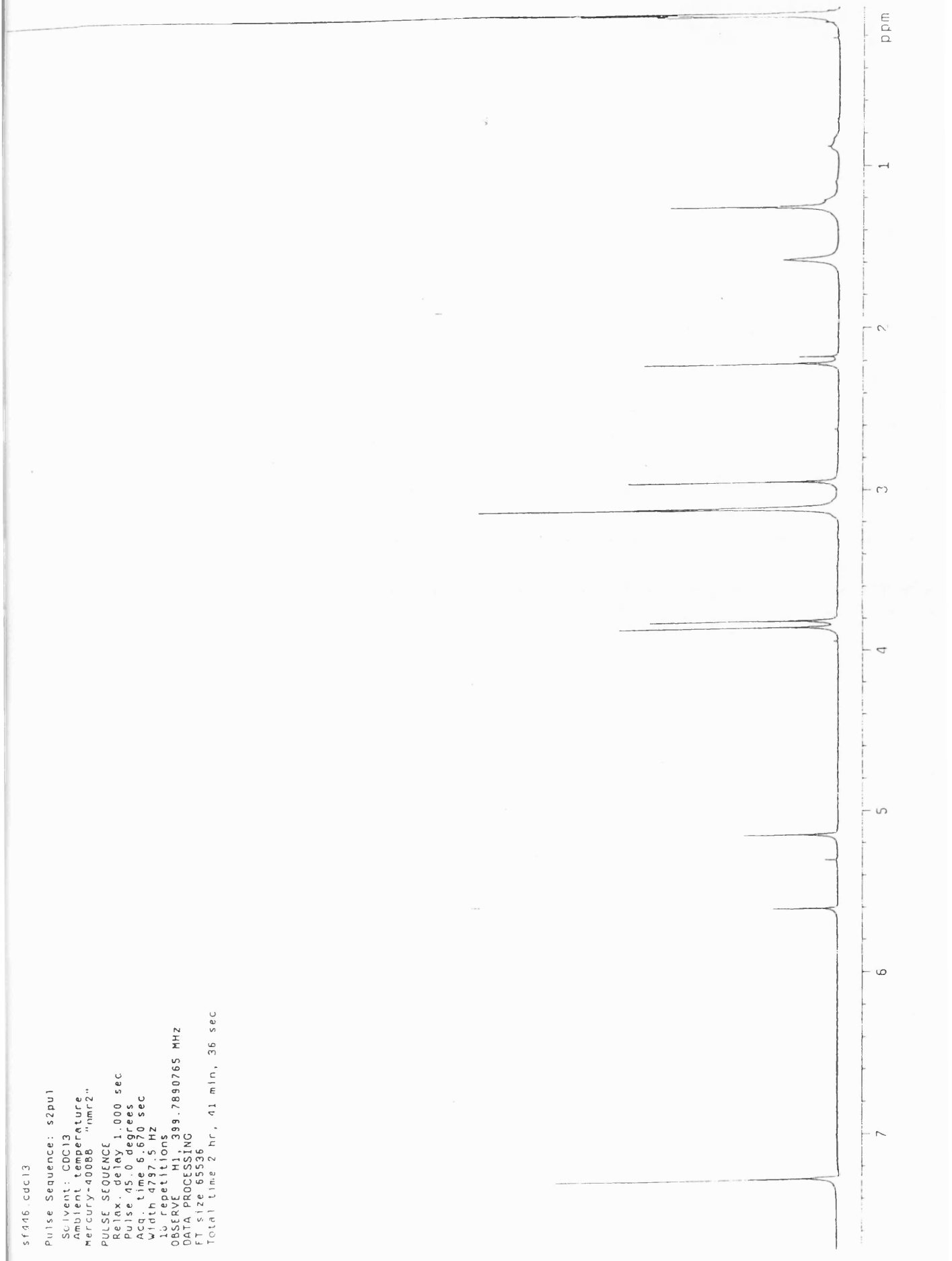
APPENDIX 13

^{1}H NMR spectrum of the mixture obtained after addition of sodium dithionite to compound **65**.

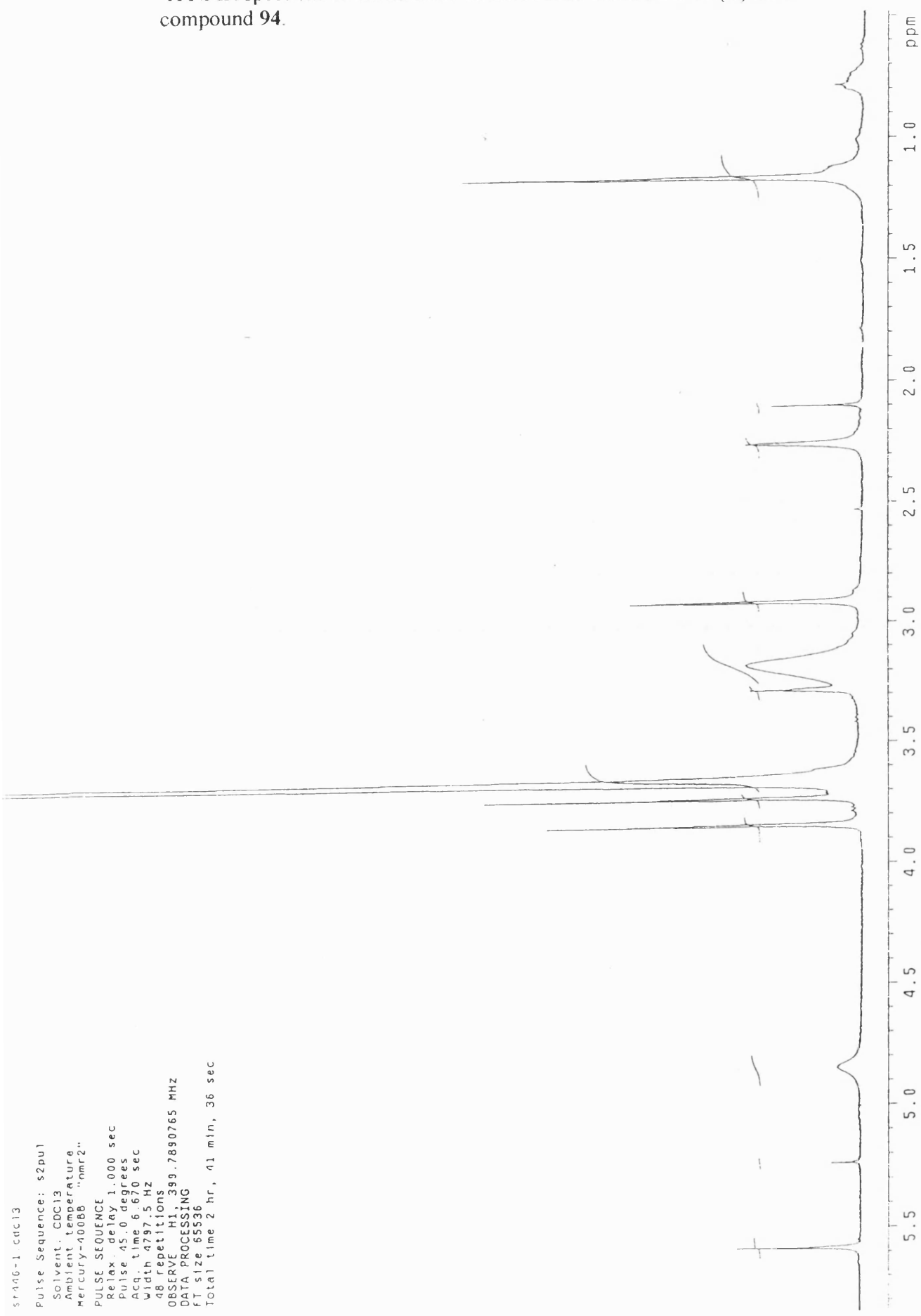
SF108-2 14939 30-d-01
Pulse Sequence: s2pul
Solvent: CD3OD
Ambient temperature
Mercury-400BB "nmr2"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.561 sec
Width 6396.7 Hz
32 repetitions
OBSERVE H1, 399.7906241 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 59 sec

APPENDIX 14

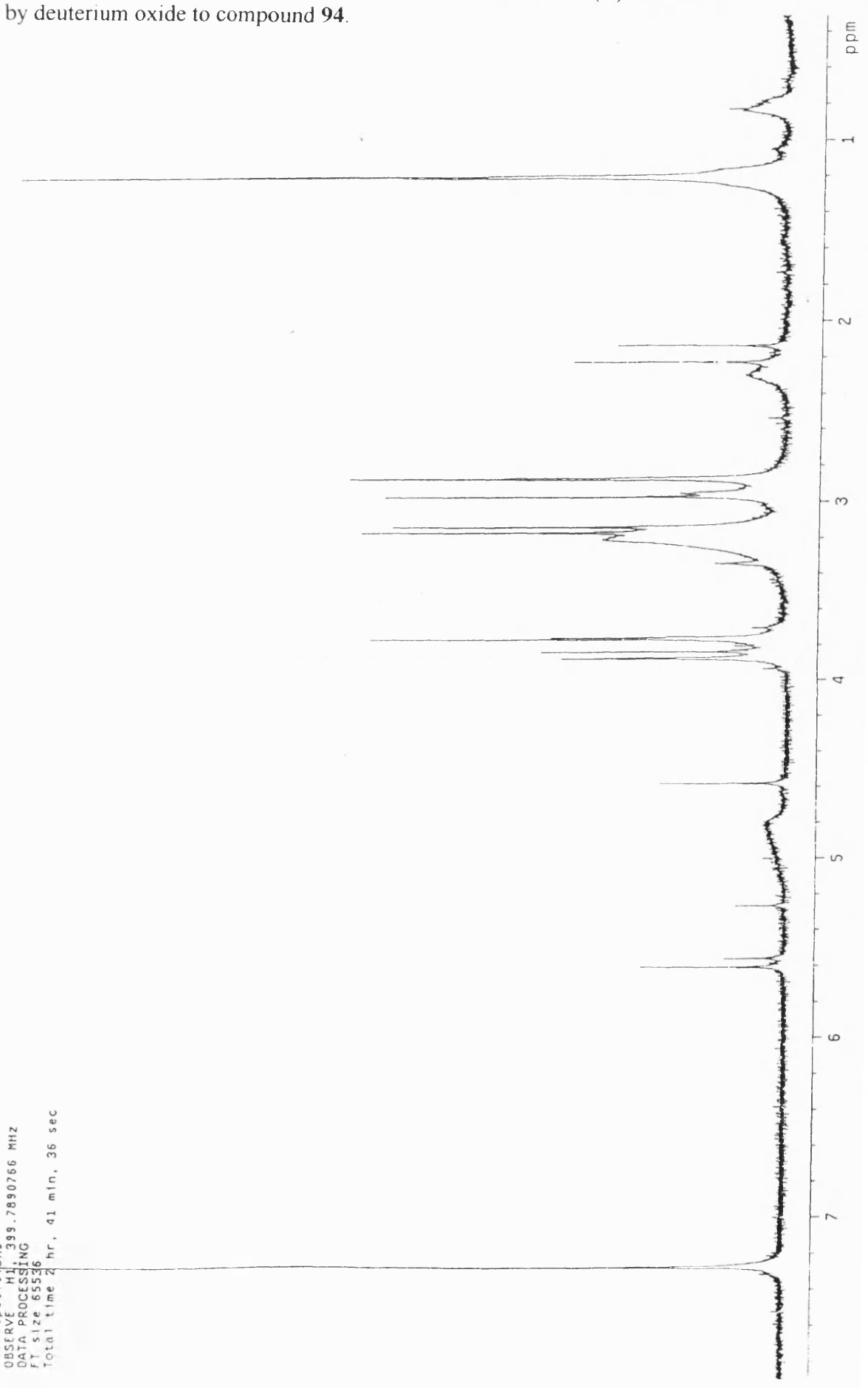
^{1}H NMR spectrum of compound **94**.



^{1}H NMR spectrum of the mixture obtained after addition of tin(II) chloride to compound **94**.

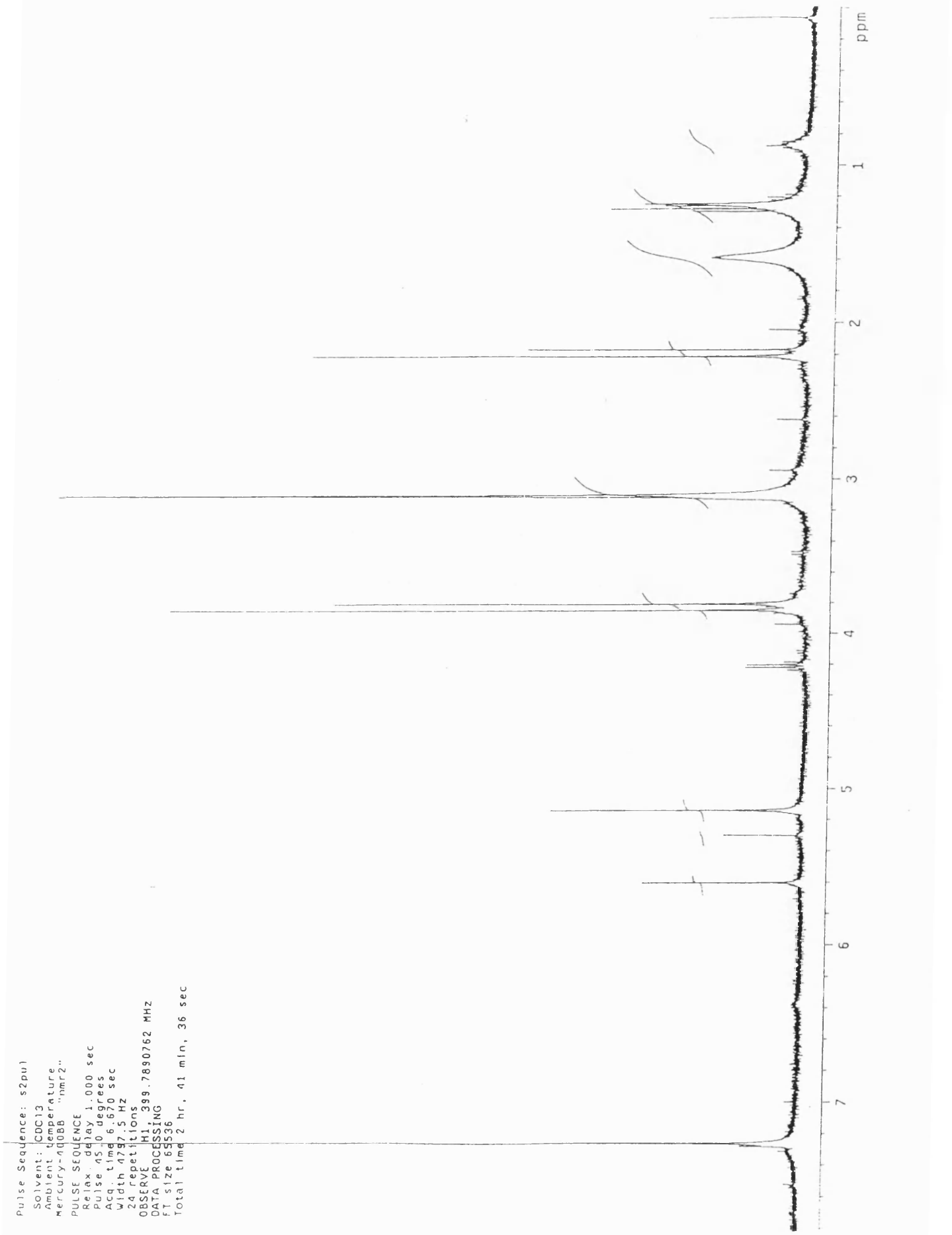


^{1}H NMR spectrum of the mixture obtained after addition of tin(II) chloride followed by deuterium oxide to compound **94**.

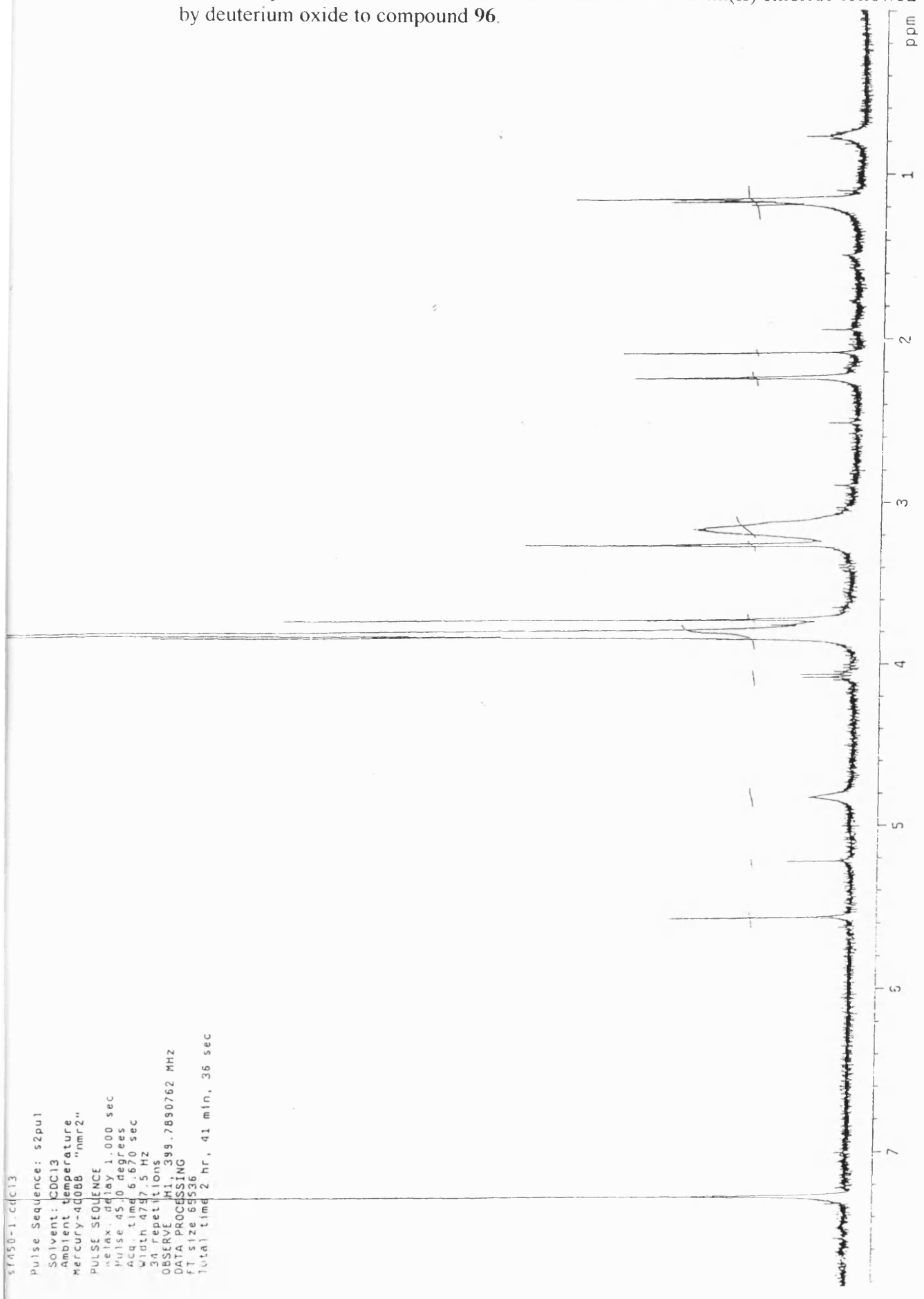


APPENDIX 17

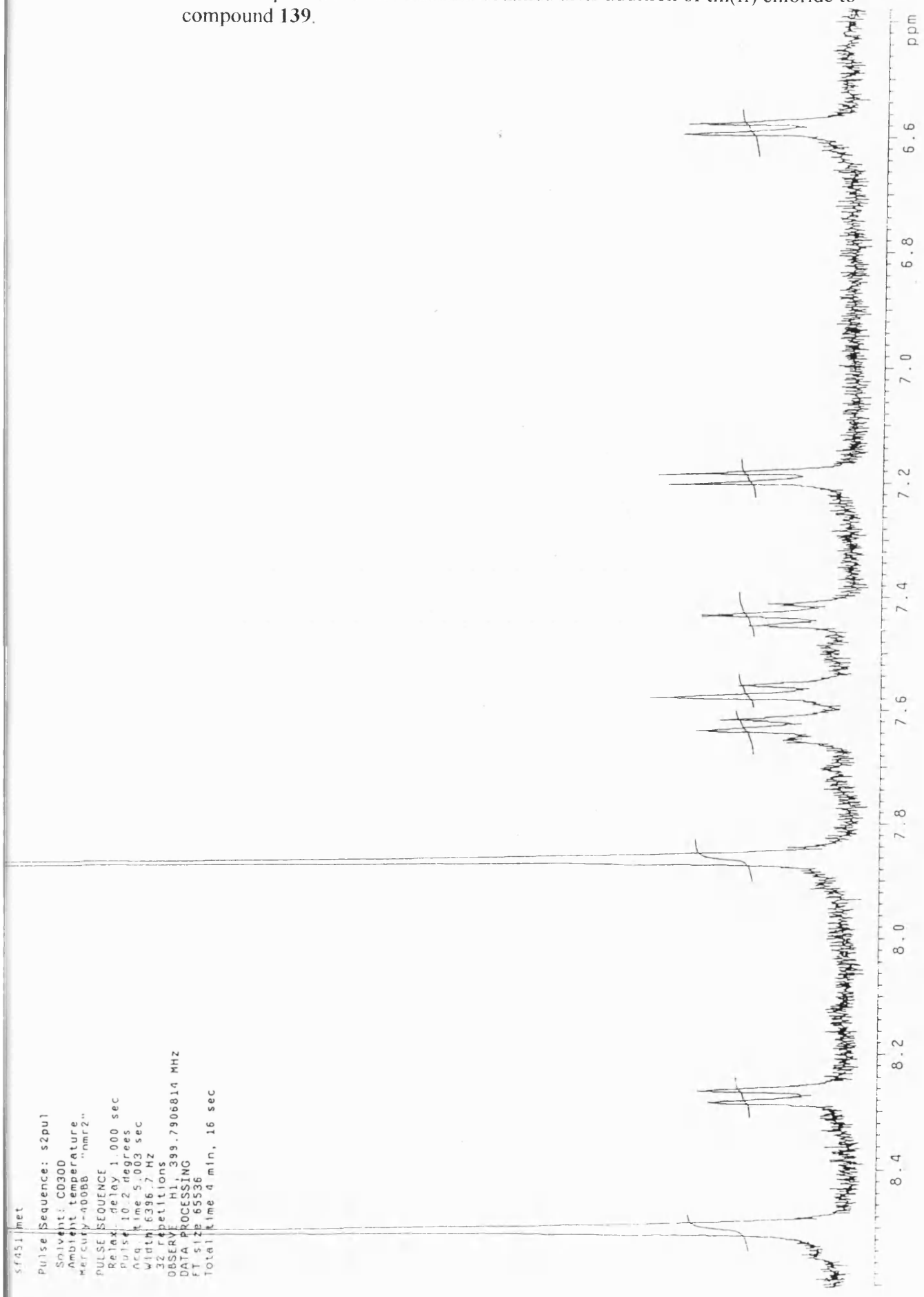
^{1}H NMR spectrum of compound **96**.



^{1}H NMR spectrum of the mixture obtained after addition of tin(II) chloride followed by deuterium oxide to compound **96**.

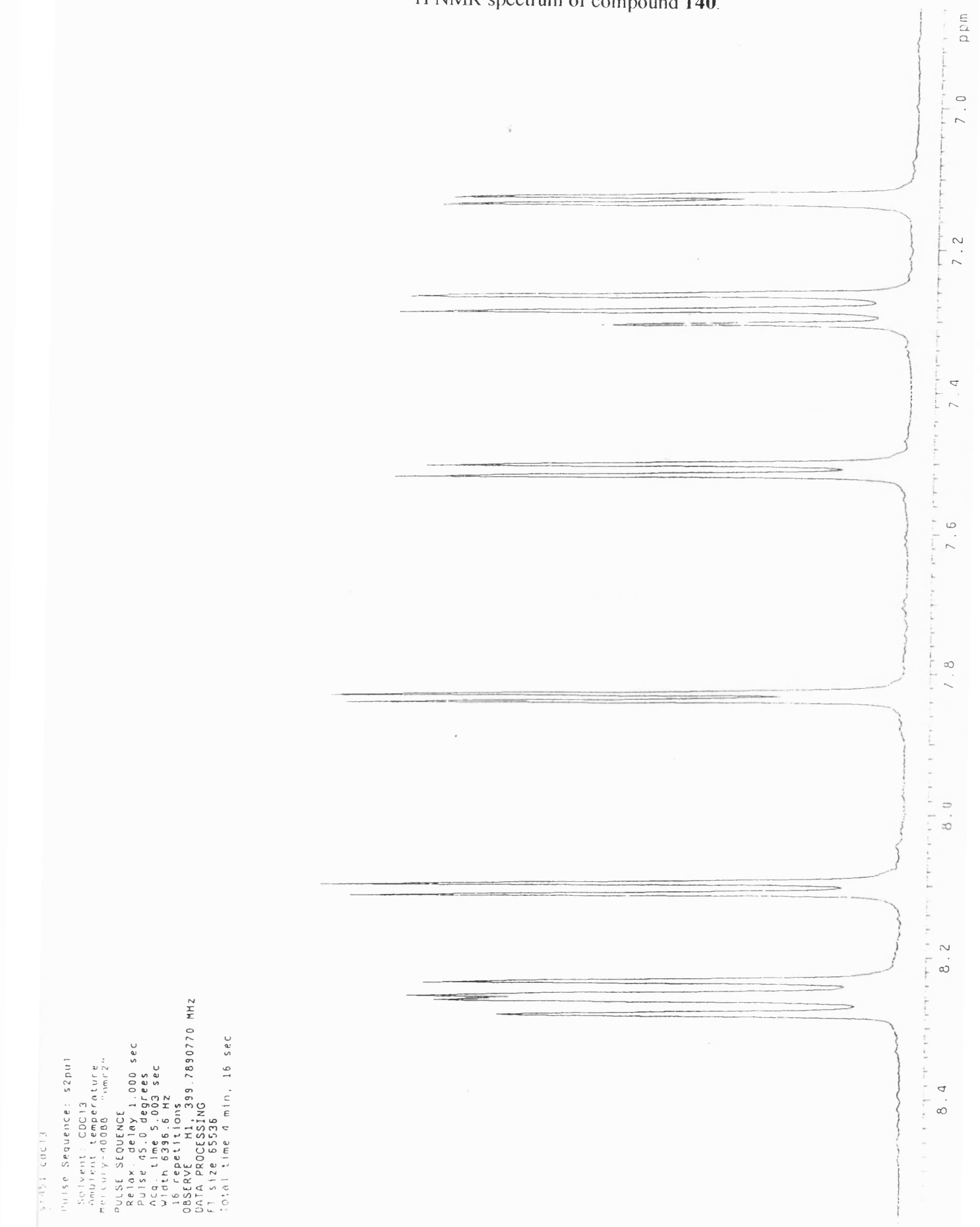


^{1}H NMR spectrum of the mixture obtained after addition of tin(II) chloride to compound **139**.



APPENDIX 20

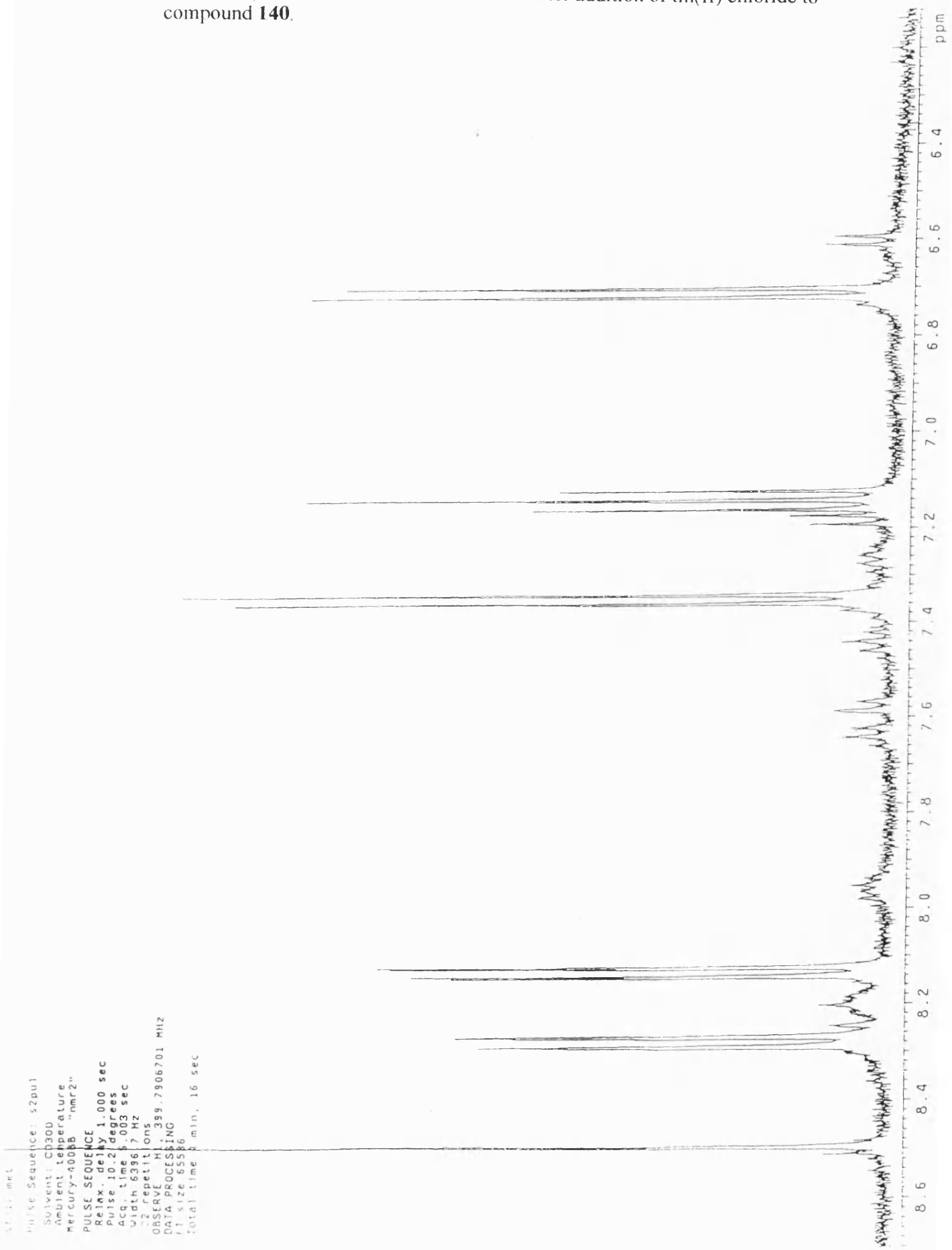
^{1}H NMR spectrum of compound **140**.



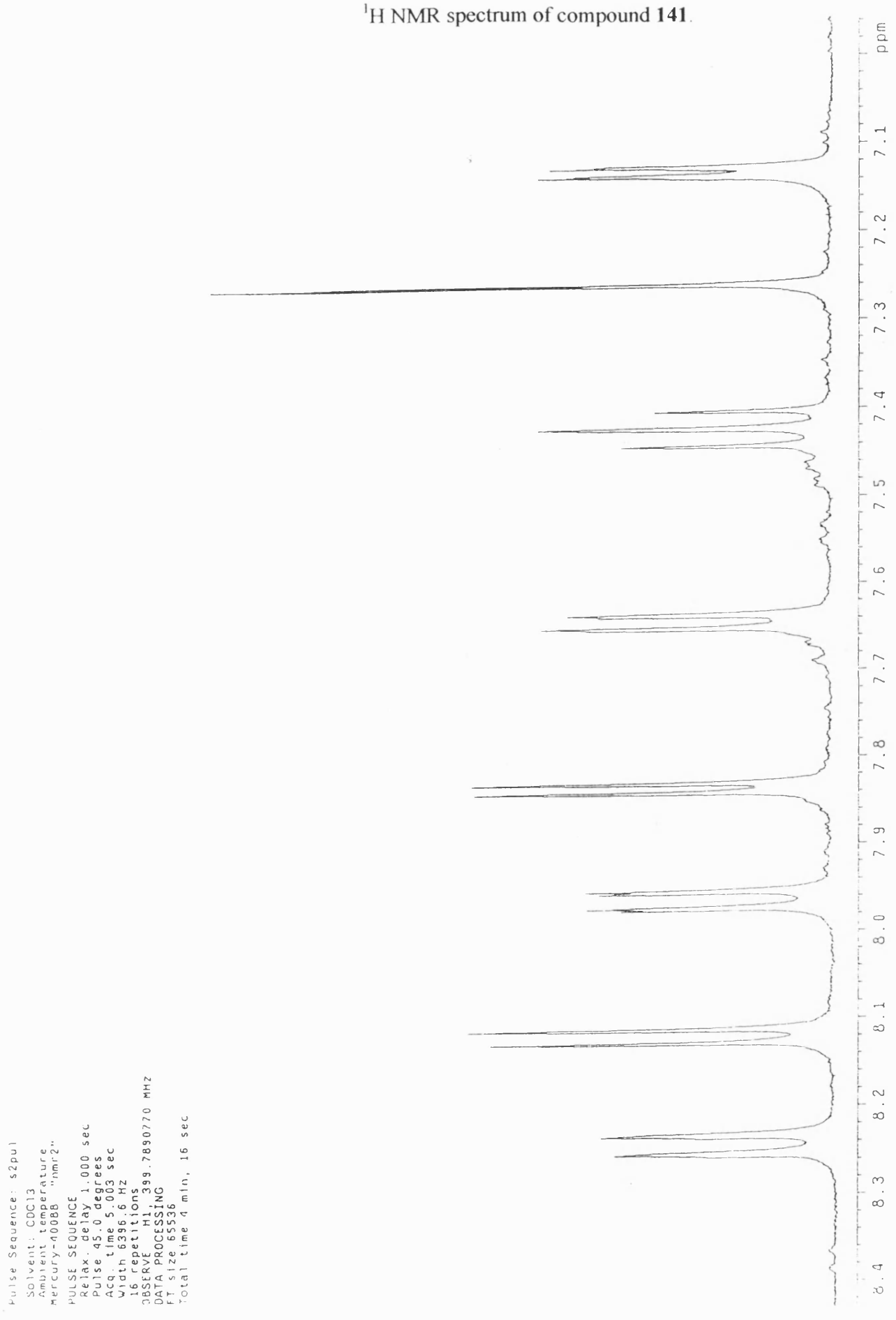
^{1}H NMR spectrum of compound **140**

APPENDIX 21

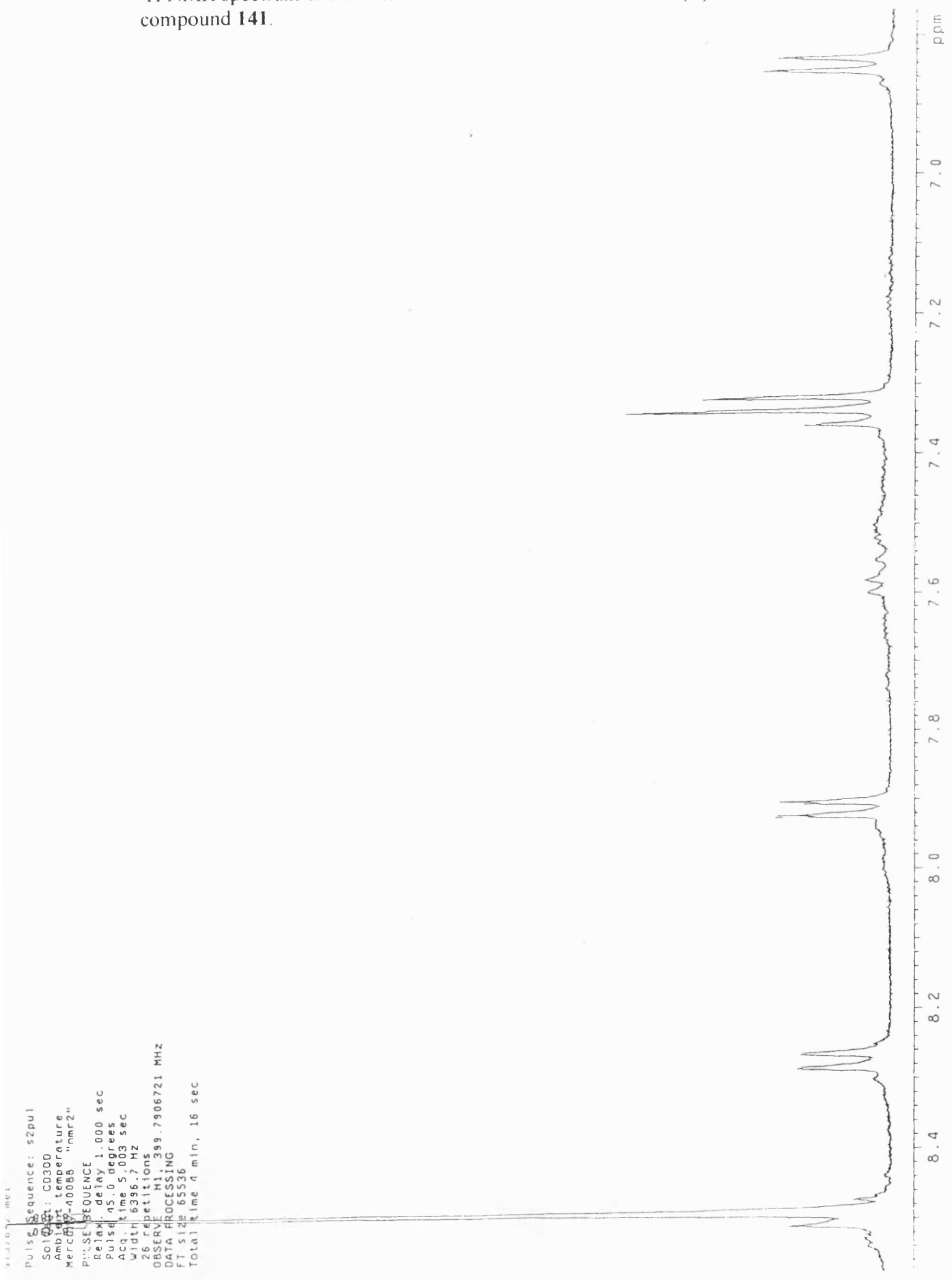
^{1}H NMR spectrum of the mixture obtained after addition of tin(II) chloride to compound **140**.



^{1}H NMR spectrum of compound **141**.



^{1}H NMR spectrum of the mixture obtained after addition of tin(II) chloride to compound **141**.

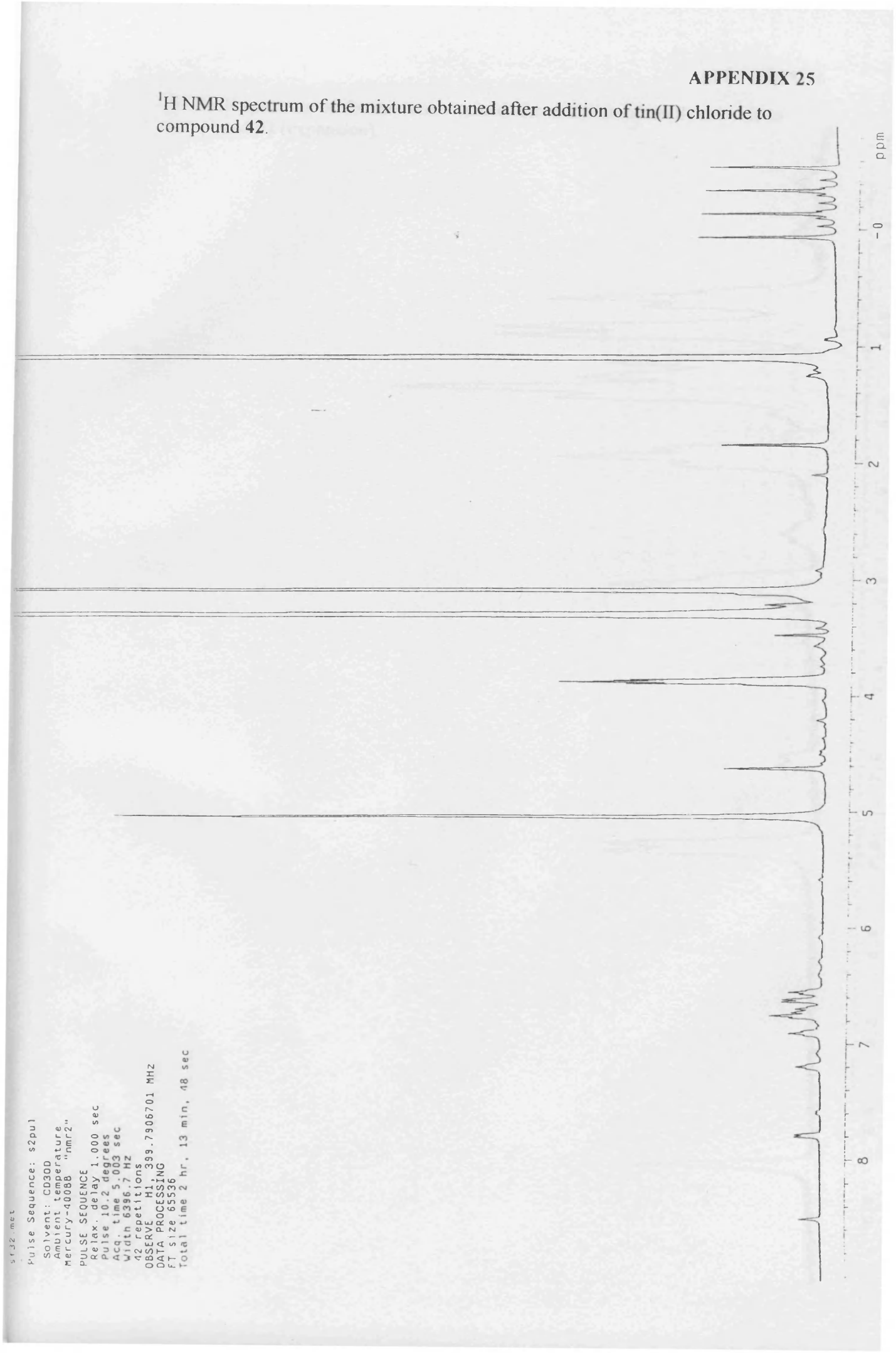


APPENDIX 24

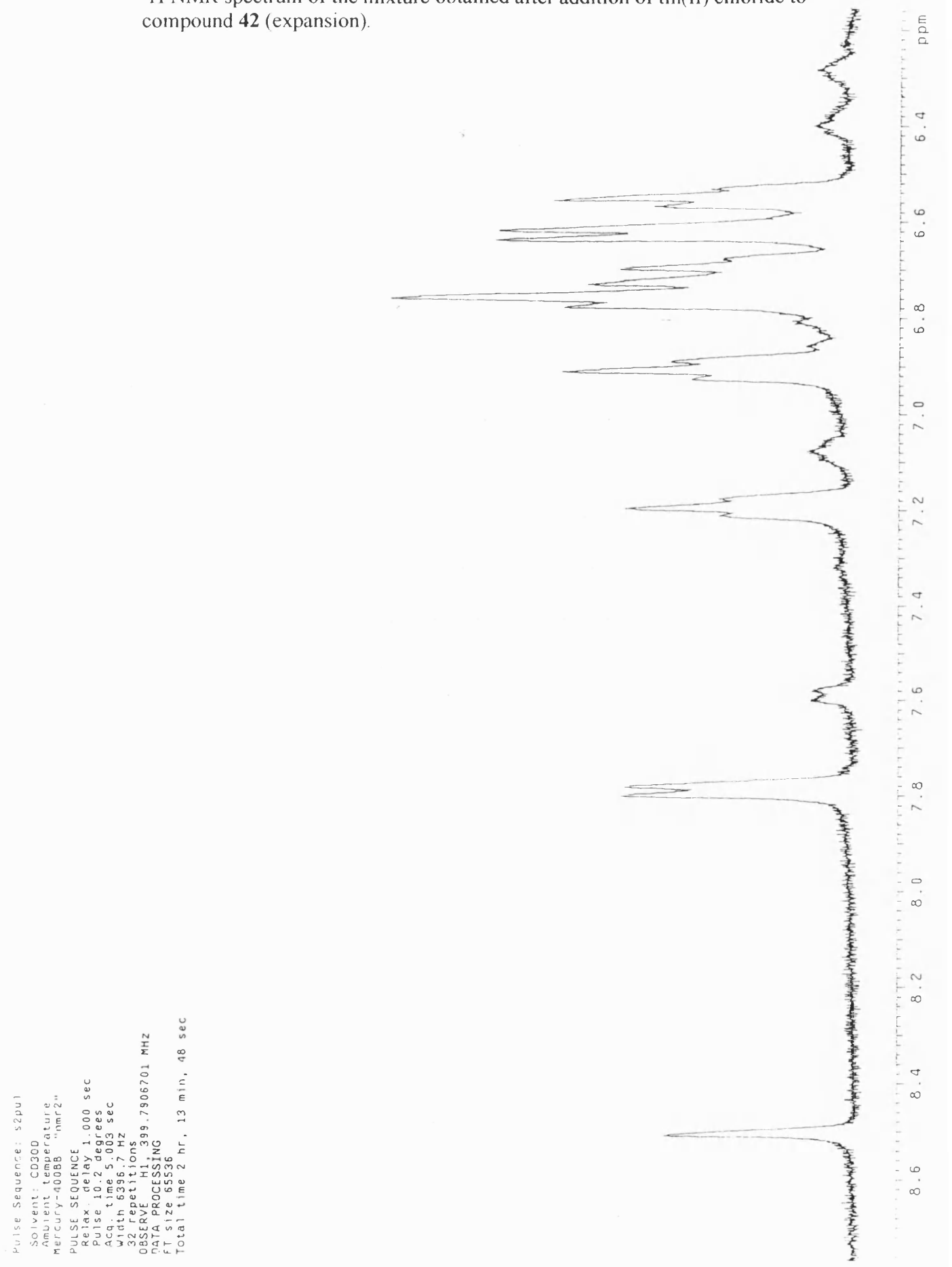
^{1}H NMR spectrum of compound **42**.

APPENDIX 25

^{1}H NMR spectrum of the mixture obtained after addition of tin(II) chloride to compound **42**.

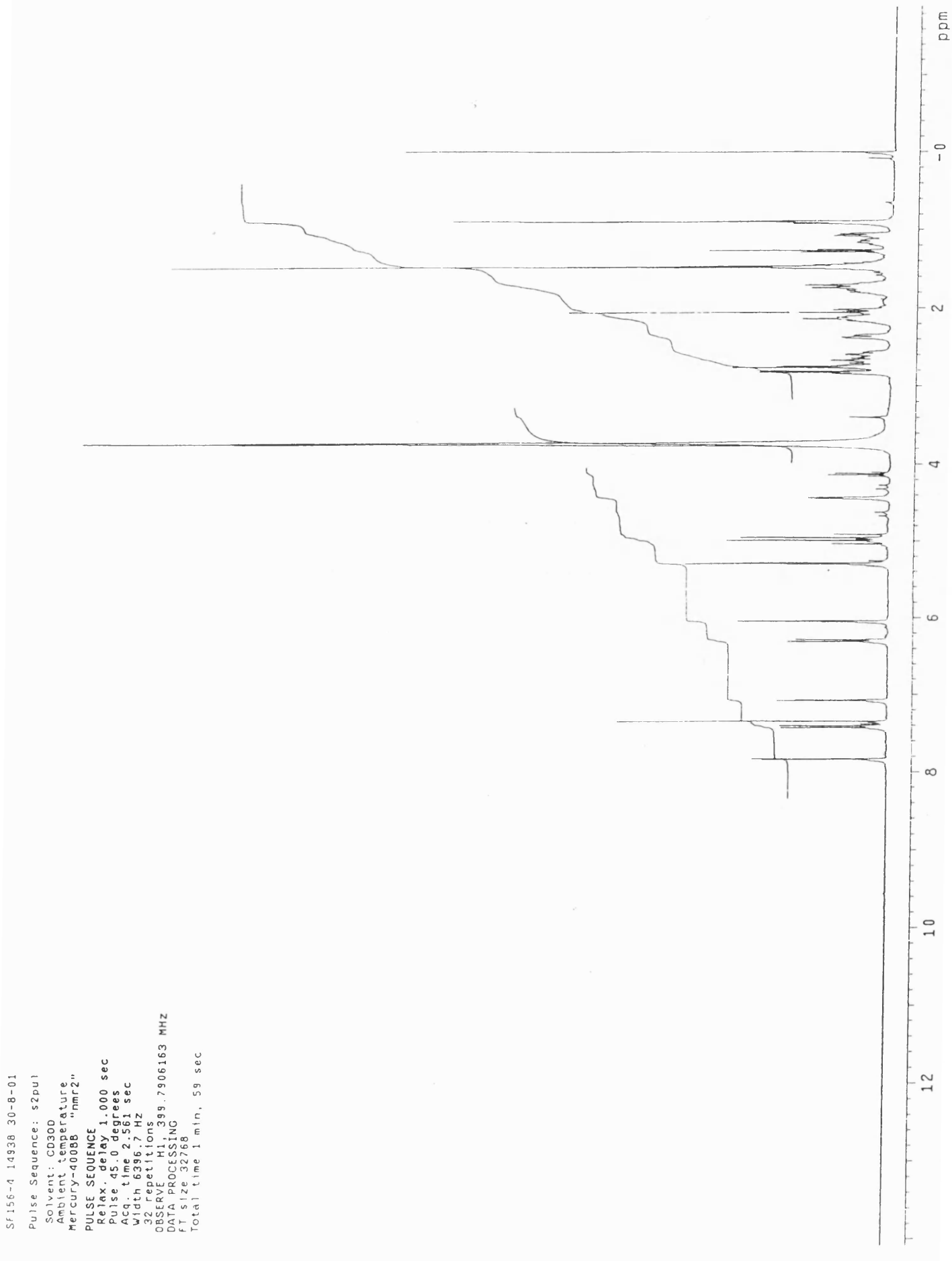


^{1}H NMR spectrum of the mixture obtained after addition of tin(II) chloride to compound **42** (expansion).



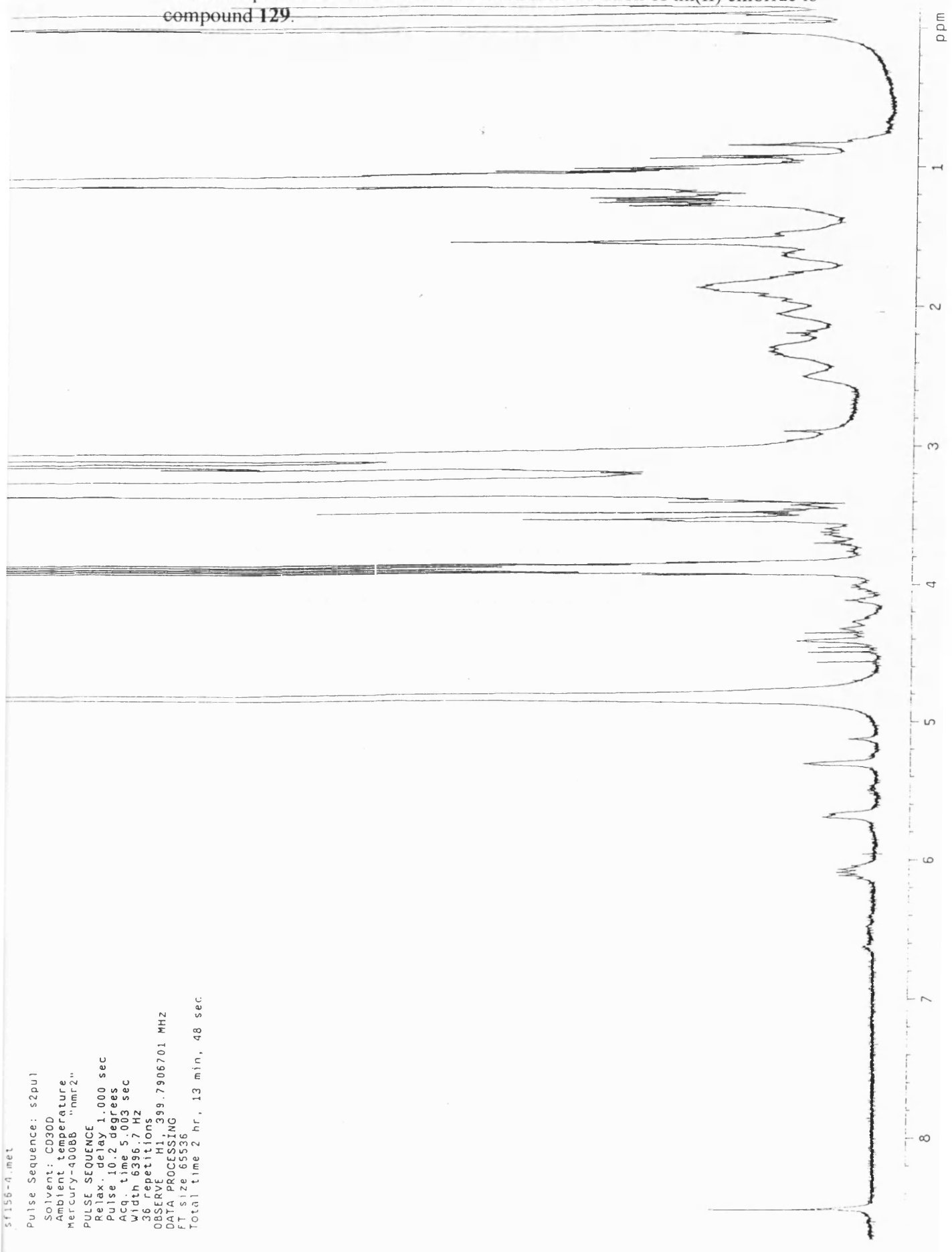
APPENDIX 27

^{1}H NMR spectrum of compound **129**.



APPENDIX 28

^{1}H NMR spectrum of the mixture obtained after addition of tin(II) chloride to ~~compound~~ **129**.



APPENDIX 29

POSTER ABSTRACT

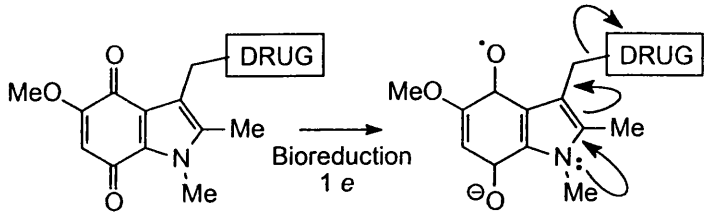
Abstract of poster presented at the 221St National Meeting of the American Chemical Society, San Diego, April 2001 and at the 11th Symposium on Medicinal Chemistry in Eastern England, Hatfield, April 2001.

Synthesis and Evaluation *in vitro* of Bioreductively Activated Prodrugs of Anti-Inflammatory Agents

Sandra Ferrer University of Bath *Supervisors*: Declan P. Naughton
Michael D. Threadgill
David R. Blake

Introduction

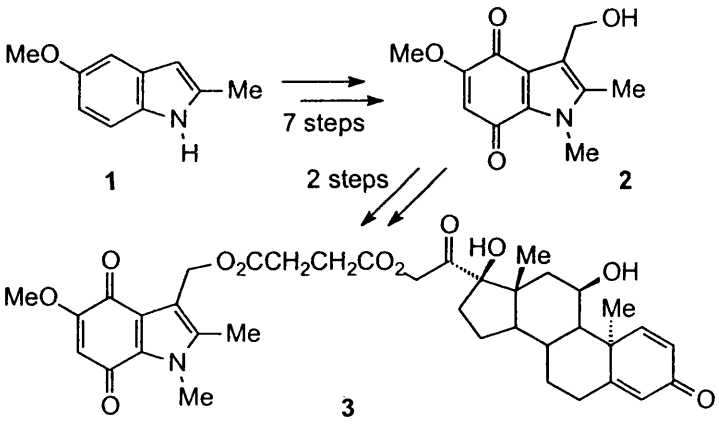
Hypoxia is present in several diseases, such as rheumatoid arthritis, osteoarthritis, some cancers and diabetes. This physiological state facilitates selective drug release from prodrugs *via* reductively activatable triggers[1], including indole-4,7-diones and nitroheterocycles. Up to 20% of the inflamed synovium is hypoxic in arthritic joints. Activities of key reductases (cytochrome P450 reductase, DT-diaphorase, cytochrome B5 reductase) are comparable to those found in tumour cell lines in which bioreductive prodrugs exhibit optimal efficacy[2].



Scheme 1. Bioreduction of indole-4,7-dione prodrug trigger and selective release of drug. in hypoxic tissue.

Discussion

The target prodrugs were indole-4,7-dione and nitrothiophene conjugates of the anti-inflammatory drugs prednisolone and aspirin. 5-Methoxy-2-methylindole (**1**) was converted to the quinone (**2**) in 7 steps, after modification and optimisation of the sequence reported by Naylor *et al*[3]. Treatment of (**2**) with $SOCl_2$ gave the chloromethylindole which was used to alkylate the carboxylate derived from prednisolone hemisuccinate to give (**3**) in moderate yield. An alternative strategy was used to generate the nitrothiophene analogue. Ring-opening of succinic anhydride with 5-nitrothiophene-2-methanol gave the succinate monoester. This was coupled to the 21-OH of prednisolone *via* the pentafluorophenyl ester in high yield. Preliminary biomimetic release studies showed rapid release of drug, using the system developed previously by us[4].



Scheme 2. Synthesis of indolequinone-prednisolone conjugate **3**.

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3. M. A. Naylor, M. Jaffar, J. Nolan, M. A. Stephens, S. Butler, K. B. Patel, S. A. Everett, G. E. Adams, I. J. Stratford, *J. Med. Chem.*, **1997**, *40*, 2335.
4. I. Parveen, D. P. Naughton, W. J. D. Whish, M. D. Threadgill, *Bioorg. Med. Chem Lett.* **1999**, *9*, 2031.

APPENDIX 30

PUBLICATION

Paper from the present work accepted for publication in the Journal of the Chemical Society, Perkin Transaction I, 2002.

N- and *O*-Alkylation of isoquinolin-1-ones in the Mitsunobu reaction: development of potential drug delivery systems

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[b] *School of Pharmacy & Biomolecular Sciences, University of Brighton, Brighton, UK BN2 4GJ*
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First published as an Advance Article on the web ??????

Regioselective methods were investigated to prepare *N*- and *O*-alkylated isoquinolin-1-ones efficiently. The predicted regioselective alkylation of the nitrogen with (hetero)-benzyl halides was complemented using (hetero)benzylic Mitsunobu electrophiles to alkylate predominantly at the oxygen. A series of drug-delivery conjugates was prepared demonstrating control over the site of alkylation. The Mitsunobu reaction provides a new approach to 1-alkoxyisoquinolines that were unavailable *via* previous harsher synthetic methods.

Introduction

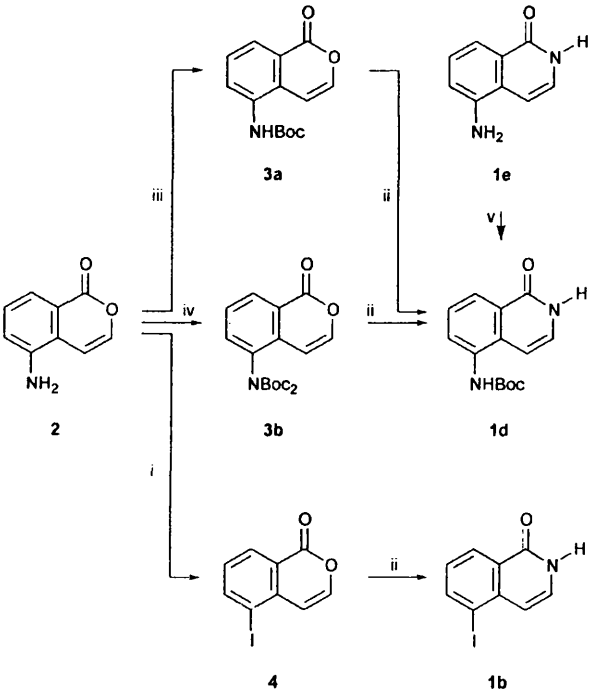
Repair and maintenance of chromosomes are, in part, controlled by the addition and removal of polymers of ADP-ribose to the DNA-binding proteins involved.[1] ADP-ribose units are added by poly(ADP-ribose)polymerase (PARP) and removed by poly(ADP-ribose)glycohydrolase (PARG). The involvement of poly(ADP-ribosyl)ation in a wide range of physiological and pathophysiological processes renders it a useful target to study biological systems and for therapeutic intervention strategies. Pathophysiological effects are mediated through overactivity of the isoform PARP-1, which depletes stores of nicotinamide adenine dinucleotide (NAD^+), the PARP substrate, leading to cell death.

5-Substituted isoquinoline-1-ones are potent inhibitors[2,3] of PARP with potential therapeutic applications in several diseases, including cancer,[4] myocardial infarction,[5] diabetes,[6] stroke,[7] rheumatoid arthritis,[8] haemorrhagic shock[3] and retroviral infections.[9] As a regulatory enzyme, PARP has beneficial roles in health and detrimental roles in disease and, therefore, inhibitors need to be targeted selectively to diseased tissues. We are developing analogues with greater aqueous solubility and prodrugs for their site-specific release. Our aim is to develop strategies to control attachment of masking prodrug units *via* either the 2-nitrogen or the exocyclic oxygen to compare the pharmacokinetic and drug release properties of these series of prodrugs.

Results and discussion

Deprotonation of isoquinolin-1-ones with base and reaction of the resulting anions with alkyl halides or tosylates are reported[10–12] to result in alkylation exclusively at nitrogen, giving 2-alkylisoquinolin-1-ones, although Kaneko *et al.*[13] note that traces (<2%) of the corresponding *O*-alkylated products (1-alkoxyisoquinolines) are also obtained in reactions with 4-bromobut-1-ene and its homologues. Interestingly, harder electrophiles, such as triflic anhydride[14] and silylating agents,[15] react at exocyclic oxygen, although the nucleophile may be the neutral molecule in these cases. 1-Alkoxyisoquinolines have generally been prepared by displacement of halides or other leaving groups from the 1-position of isoquinolines with nucleophilic alkoxides,[14,16] although the range of groups that can be introduced is limited to simple examples by the harsh conditions necessary.

Several routes were employed for the synthesis of the starting isoquinoline-1-ones used in this study (Scheme 1). Isoquinolin-1-one **1a** ("isocarbostyril") is commercially available. Our previously reported synthesis[11] of 5-iodoisoquinolin-1-one **1b**, by Curtius rearrangement of 2′-iodocinnamoyl azide and cyclisation of the intermediate 2-(2-iodophenyl)ethenyl isocyanate at



Scheme 1 Synthetic approaches to 5-iodoisoquinolin-1(2*H*)-one **1b** and 5-(Boc-amino)isoquinolin-1(2*H*)-one **1d**. *Reagents and conditions*: i, $NaNO_2$–aq. HCl–KI, 0 °C; ii, NH_3–$MeOCH_2CH_2OH$, reflux; iii, Boc_2O (1.5 equiv.)–Et_3N–CH_2Cl_2–DMF; iv, Boc_2O (3.0 equiv.)–Et_3N–CH_2Cl_2–DMF; v, Boc_2O (3.0 equiv.)–Et_3N–DMF.

DOI: 10.1039/b109776h

280 °C, proved not to be amenable to the routine preparation of large quantities and a new route was required. Following the newer route to **1b**, 5-aminoisocoumarin **2** was diazotised and the diazonium group was replaced with iodine using potassium iodide; 5-iodoisocoumarin **4** was obtained in good yield (Scheme 1). Conversion to the isoquinolin-1-one **1b** was achieved in the usual manner, in a reliable 48% yield from **2**. 5-Bromoisoquinolin-1-one **1c** was prepared by diazotisation and Sandmeyer reaction of 5-aminoisocoumarin **2**, followed by replacement of the ring oxygen with nitrogen by treatment with ammonia at high temperature, as described previously.[17]

5-Aminoisoquinolin-1(2*H*)-one **1e** is a particularly effective inhibitor of PARP *in vivo*[3] but, to be able to study the nucleophilic reactivity of the 2-nitrogen and the exocyclic oxygen, the potentially more nucleophilic 5-amine required protection. In the first route to **1e**, the Boc-amino unit was assembled on the isocoumarin prior to conversion to the isoquinolinone (Scheme 1). Reaction of 5-aminoisocoumarin **2** with a small excess of di-*tert*-butyl dicarbonate led to the mono-Boc compound **3a**, whereas treatment with three equivalents gave the *N*,*N*-diBoc analogue **3b** in good yield. As expected, **3a** was converted to the mono-Boc isoquinolinone **1d** with ammonia in refluxing 2-methoxyethanol. When **3b** was subjected to the same conditions, not only was the isocoumarin converted to the isoquinolinone but also one of the Boc groups was ammonolysed from the 5-amino function, also giving the mono-Boc isoquinolinone **1d** efficiently. Direct introduction of Boc protection to 5-aminoisoquinolin-1(2*H*)-one **1e** also gave **1d** but in lower yield.

In model studies, (Scheme 2), the reactions of the isoquinolin-1(2*H*)-ones **1** with simple benzyl electrophiles were investigated. The 5-unsubstituted isoquinolinone **1a** was deprotonated with sodium hydride and the anion reacted with benzyl bromide to give the *N*-benzylated compound **6a** as the only isolable product.[11] Generation of the anions from the 5-haloisoquinolinones **1b,c** with the soluble non-nucleophilic base lithium bis(trimethylsilyl)amide was technically more facile. Again, alkylation with the benzyl chlorides **5b,c** gave only the products of *N*-alkylation, **6b,c**, respectively.[11] In contrast, reaction of **1a** with benzyl alcohol under Mitsunobu conditions gave only the *O*-benzylated product **7**.

Extension of the study to *pseudo*benzylic 5-membered ring heterocycles produced a similar outcome. The anion derived from isoquinolinone **1a** reacted only at nitrogen[11] with 2-chloromethylfuran **8a** and 5-nitrofuran-2-yl tosylates **8b**, affording the isoquinolinones **9a,b**. The yield of the nitrofuranylmethylisoquinolinone was low, probably owing to side-reactions involving the relatively acidic methylene protons in **8b**. Similarly, the anion of **1a** reacted with 2-chloromethylthiophene **8c** only at nitrogen, giving **9c**. However, this anion only served to degrade the corresponding nitrothiophene electrophile 2-chloromethyl-5-nitrothiophene;[18] the methylene protons are presumably even more acidic in these molecules than are those in **8b**. In this series, the Mitsunobu alkylations were carried out using (5-nitro-2-thienyl)methanol **8d** as the electrophile; this represents a sterner test of the applicability of the process, owing to the possibility of reduction of the nitro group by the triphenylphosphine. Reaction of the isoquinolinones **1a–c** with **8d** under the standard Mitsunobu conditions, however, led to the *O*-alkylated products **10a–c**, respectively, in moderate yields but without any trace of formation of *N*-alkylated materials.

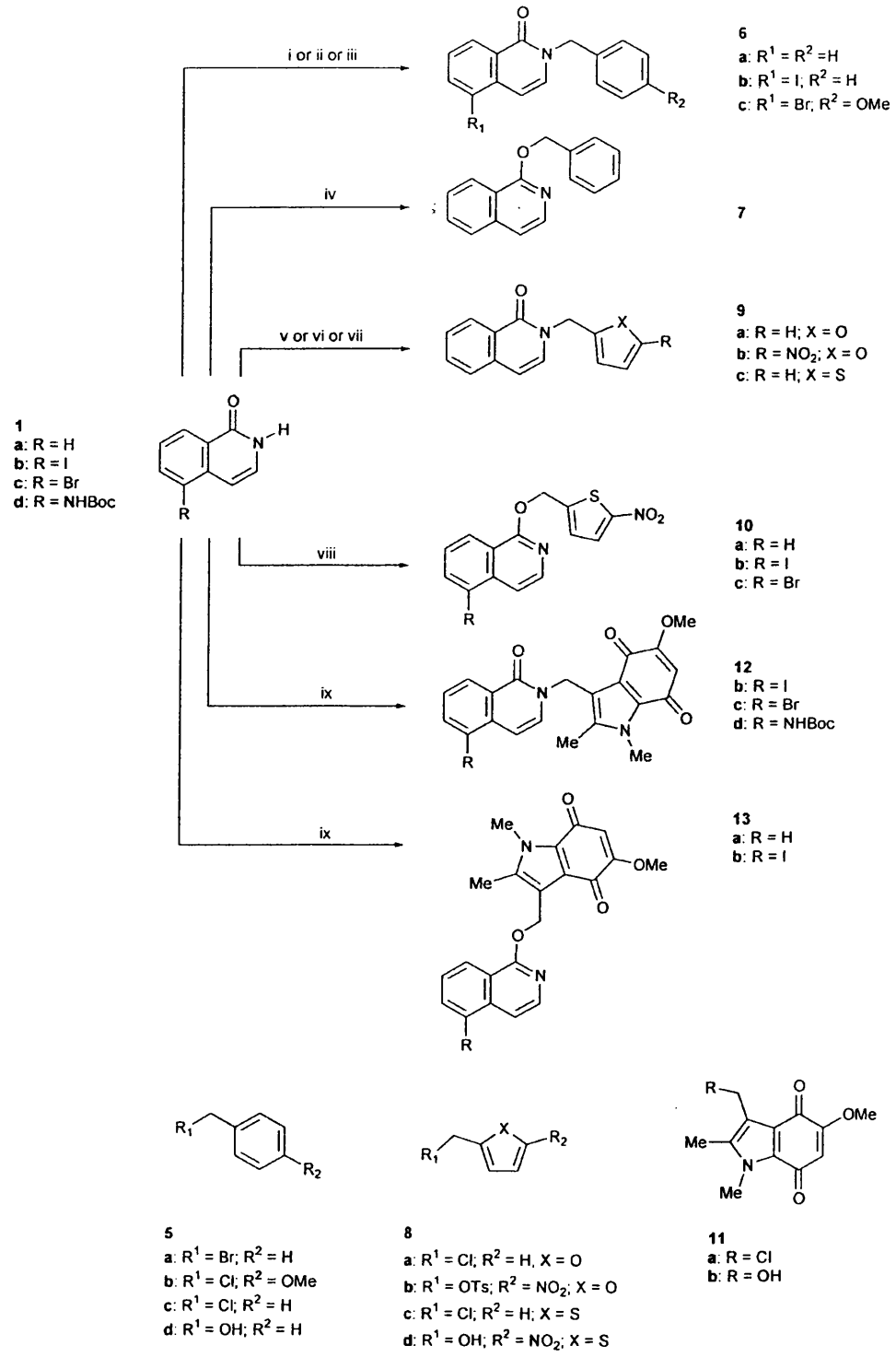
Thus, in the cases of the monocyclic benzylic electrophiles **5** and **8**, the outcomes of the reactions are clearly defined: conventional alkylation with benzylic halides and tosylates gives only reaction at nitrogen, whereas the Mitsunobu conditions give exclusively the *O*-alkylated products. With the bicyclic electrophiles **11** based on the 4,7-dioxoindole-3-methylene unit, however, the outcomes are more subtle. All attempts to alkylate the anions derived from the isoquinolinones **1a–c** with the chloromethyl-1*H*-indole-4,7-dione **11a**[19] failed. The indoledione unit was destroyed under the basic reaction conditions; the isoquinolinone anions are clearly much more basic than are the phenolate anions which are reported[19] to react smoothly with this reagent. Naylor *et al.*[19] observed solvent-dependent regioselectivity in alkylation of 2-fluorophenolate and 4-fluorophenolate, ethyl acetate favouring *C*-alkylation and DMF favouring *O*-alkylation. When the isoquinolinones **1a–d** were treated with the corresponding alcohol **11b** under Mitsunobu conditions, both the *N*-alkylated isoquinolinones **12** and the 1-alkoxyisoquinolines **13** were obtained, although never as mixtures. In repeated experiments, the 5-unsubstituted isoquinolinone **1a** only reacted at oxygen, giving **13a**. In contrast, the 5-bromo- and 5-Boc-amino analogues **1c,d** gave only the *N*-linked compounds **12c,d**. Most surprisingly, the 5-iodoisoquinolin-1(2*H*)-one **1b** gave the *N*-linked product **12b** and the *O*-linked product **13b** not as a mixture but as the sole isolable products from different experiments under apparently the same conditions.

Distinction between the structures of the *N*-alkylisoquinolinones **6**, **9**, **12** and 1-alkoxyisoquinolines **7**, **10**, **13** was made primarily on the basis of their ^{1}H and ^{13}C NMR spectra (Table 1). Taking the isomeric pair **6a** and **7**, the NCH_2 protons of **6a** resonate at δ 5.20, whereas the OCH_2 proton signal of **7** appears downfield at δ 5.58, due to the greater electronegativity of oxygen. Significant changes in chemical shift are also seen for two of the isoquinoline protons. In **6a**, the 3-H resonates at δ 7.06 and the 4-H at δ 6.46; in **7**, the corresponding signals are at δ 8.00 and δ 7.25, respectively, reflecting the enamide character of the isoquinolin-1(2*H*)-one and the greater aromaticity of the 1-alkoxyisoquinoline. The signals of the protons of the carbocyclic rings are relatively similar for the two compounds and are not diagnostic. Using this guide, **6b,c** can be identified as *N*-benzylisoquinolinones in showing chemical shifts for the CH_2 protons and for 3-H and 4-H as does **6a**. Further confirmation of the structure of **6b** was given by an unequivocal synthesis, as shown in Scheme 3. 5-Bromoisocoumarin **14** (prepared as described previously by us[17]) was condensed with 4-methoxybenzylamine to give **6b** in modest yield. Since the methoxybenzyl unit was attached to nitrogen in the reagent, the product of this reaction must be 5-bromo-*N*-(4-methoxybenzyl)isoquinolin-1(2*H*)-one **6b**, rather than any *O*-linked isomer. The general pattern of proton chemical shifts was similar for the furans **9a,b** and the thiophenes **9c**, **10a–c**. In the *N*-linked compounds **9**, the CH_2 protons resonated in the range δ 5.1–5.4 and in the *O*-linked **10**, the corresponding signals appeared at δ *ca.* 5.7. In **9**, the isoquinoline 3-H

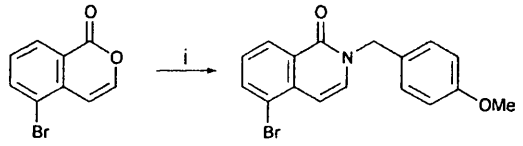
Table 1 Selected NMR chemical shifts[a] for the *N*-alkylisoquinolinones and 1-alkoxyisoquinolines

Compound	δ_H (CH_2)	δ_C (CH_2)	δ_H (3-H)	δ_H (4-H)
6a	5.20[b]	51.6[b]	7.06[b]	6.46[b]
6b	5.22[b]	51.9[b]	7.19[b]	6.72[b]
6c	5.15	[c]	7.18	6.82
7	5.58	[c]	8.00	7.25
9a	5.17[b]	44.2[b]	7.14[b]	6.45[b]
9b	5.23[b]	[c]	7.24[b]	6.58[b]
9c	5.34	[c]	7.5	6.49
10a	5.74	62.3	8.00	7.30
10b	5.74	62.8	8.10	7.49
10c	5.75	62.9	8.12	7.65
12b	5.29	32.6	7.80	6.66
12c	5.30	32.6	7.82	6.77
12d	5.25	[c]	7.47	6.57
13a	5.72	53.4	8.00	7.20
13b	5.71	56.4	8.08	7.40

[a] Spectra recorded of solutions in $CDCl_3$. [b] Data taken from [ref. 10]. [c] Not determined.



Scheme 2 Studies on the *N*- and *O*-alkylation of isoquinolin-1(2*H*)-ones **1a–d**. *Reagents*: i, NaH–DMF–**5a**; ii, LiN(SiMe$_3$)$_2$–THF–**5b**–NaI; iii, LiN(SiMe$_3$)$_2$–THF–**5c**–NaI; iv, Ph$_3$P–diethyl azodicarboxylate (DEAD)–**5d**–THF; v, LiN(SiMe$_3$)$_2$–THF–**8a**–NaI; vi, NaH–DMF–**8b**; vii, LiN(SiMe$_3$)$_2$–THF–**8c**; viii, Ph$_3$P–DEAD–**8d**–THF; ix, Ph$_3$P–DEAD–**11b**–THF.



14 **6c**

Scheme 3 Unequivocal synthesis of 5-bromo-2-(4-methoxybenzyl)isoquinolin-1(2*H*)-one **6c**. *Reagents and conditions*: i, 4-MeOBnNH$_2$, MeOCH$_2$CH$_2$OH, reflux.

and 4-H signals were at δ 7.1–7.5 and δ 6.4–6.6, whereas in **10**, the signals were at δ 8.0–8.1 and δ 7.3–7.7, respectively. Clearly the range of chemical shifts for 4-H in **10** is greater than that for 3-H, as the former is expected to be more influenced by the nature of the 5-substituent than is the latter. Similarly, in the indole-4,7-dione series **12** and **13**, highly diagnostic trends in chemical shift were observed. In these series, the nature of the *O*- or *N*-substituent is constant, so the chemical shifts should be more directly comparable. Hence, the resonances for the CH$_2$ protons of **12b–d** fall in the very narrow range δ 5.25–5.30 and

the corresponding signals for **13a,b** are at δ 5.72 and δ 5.71, respectively. As expected, the chemical shifts of 3-H and 4-H are sensitive to the nature of the 5-substituent. The chemical shifts in the ^{13}C NMR spectra show analogous trends, although the ranges are wider and sometimes overlapping. In the *N*-linked isoquinolinones **6**, **9**, **12**, the $^{13}CH_2$ peaks are in the range δ 32–52; whereas, in the *O*-linked isoquinolines **10**, **13**, the corresponding range is δ 53–63.

In this paper, we have reaffirmed the regioselectivity of alkylation of isoquinolin-1-one anions with (hetero)benzyl halides as taking place essentially exclusively at nitrogen. In contrast, reaction with benzylic and heterobenzylic Mitsunobu electrophiles takes place at oxygen in most cases. In some cases, the outcome of the reaction is extremely sensitive to the reaction conditions and to the nature of the 5-substituent of the isoquinolin-1-one; mixtures of products were never observed. Although Mitsunobu reactions with isoquinolin-1-ones as the nucleophilic component are previously unreported, Manhas *et al.*[20] achieved reaction of quinazolin-4-ones with steroid alcohols through the exocyclic oxygen of the heterocycles. These observations are consistent with regioselectivity being controlled by the hardness/softness of the nucleophiles and electrophiles involved. Such control is also evident in the *O*-selective alkylation of amides and the *S*-selective alkylation of thioamides with alcohols under Mitsunobu conditions;[21] phthalimide, of course, is alkylated at nitrogen in a synthetically useful approach to primary amines.[22] The Mitsunobu electrophile derived from the 4,7-dioxoindol-3-ylmethanol **11b** must be very close to matching hardness with the O and N nucleophilic centres of the isoquinolinone anions.

The Mitsunobu reaction is shown here to offer a potential approach to 1-alkoxyisoquinolines carrying useful functionality in the 1-substituent; such compounds are not available through previous harsh synthetic methods. The 2-(4,7-dioxo-1*H*-indol-3-ylmethyl)isoquinolin-1(2*H*)-ones and 1-[(4,7-dioxo-1*H*-indol-3-yl)methoxy]isoquinolines may represent a new class of prodrugs to target PARP inhibitors to hypoxic regions of solid tumours[17] and other diseased tissues.

Experimental

NMR spectra were recorded on samples in $CDCl_3$, unless otherwise stated. Mass spectra were obtained by fast atom bombardment (FAB) in the positive ion mode, unless otherwise stated. The stationary phase for chromatography was silica gel. Melting points are uncorrected. Solutions in organic solvents were dried with $MgSO_4$. Solvents were evaporated under reduced pressure. Experiments were conducted at ambient temperature, unless otherwise stated. The brine was saturated. DEAD refers to diethyl azodicarboxylate. DMF refers to dimethylformamide.

5-Iodoisoquinolin-1(2*H*)-one 1b

Compound **4** (300 mg, 1.1 mmol), in 2-methoxyethanol (50 cm^3), was saturated with ammonia for 1 h after which the mixture was boiled under reflux for 24 h. Evaporation yielded the isoquinolinone **1b** as pale buff crystals (200 mg, 68%): mp 239–242 °C (lit.[11] mp 238–244 °C); δ_H 6.67 (1 H, d, J = 7.4 Hz, 4-H), 7.13 (1 H, d, J = 7.4 Hz, 3-H), 7.17 (1 H, t, J = 7.5 Hz, 7-H), 8.15 (1 H, dd, J = 7.5, 1.1 Hz, 6-H), 8.35 (1 H, dd, J = 7.5, 1.1 Hz, 8-H) and 11.20 (1 H, s, NH).

5-(1,1-Dimethylethoxycarbonylamino)isoquinolin-1(2*H*)-one 1d

Method A. Compound **3a** was treated with ammonia, as in Method B, to give **1d** (76%) with properties as below.

Method B. Compound **3b** (68 mg, 0.19 mmol), in 2-methoxyethanol (10 cm^3), was saturated with NH_3 for 1 h after which the mixture was boiled under reflux for 24 h. Evaporation and chromatography (ethyl acetate–hexane 1 : 1) gave **1d** (35 mg, 71%) as pale yellow crystals: mp >230 °C (Found: C, 63.5; H, 6.28; N, 10.58. $C_{14}H_{16}N_2O_3 \cdot 0.25\ H_2O$ requires C, 63.60; H, 6.22, N, 10.20%); ν_{max} (KBr)/cm^{-1} 3320, 1690 and 1670; δ_H ($CDCl_3$) 1.54 (9 H, s, Bu^t), 6.58 (1 H, d, J 7.3, 4-H), 6.61 (1 H, brs, N*H*Boc), 7.22 (1 H, d, J 7.3, 3-H), 7.49 (1 H, t, J = 8.1 Hz, 7-H), 8.04 (1 H, d, J = 8.1 Hz, 6-H), 8.22 (1 H, d, J = 8.1 Hz, 8-H) and 10.60 (1 H, brs, NH); *m/z* 261.1235 (M + H) ($C_{14}H_{17}N_2O_3$ requires 261.1239) and 260.1169 (M) ($C_{14}H_{16}N_2O_3$ requires 260.1161).

Method C. Triethylamine (1.8 g, 18 mmol) was added to 5-aminoisoquinolin-1(2*H*)-one **1e**[3] (600 mg, 3.7 mmol) in DMF (40 cm^3) and the solution was cooled to 0 °C. Di-*tert*-butyl dicarbonate (2.45 g, 11.2 mmol) was added in portions during 2 d. The evaporation residue, in ethyl acetate, was washed with water and brine and was dried. Evaporation and chromatography (ethyl acetate–hexane 1 : 4) gave **1d** (0.57 g, 60%) as a pale yellow powder with properties as above.

5-(1,1-Dimethylethoxycarbonylamino)isocoumarin 3a

To 5-aminoisocoumarin[17] **2** (700 mg, 4.4 mmol) in dichloromethane (3.0 cm^3) was added DMF (3.5 cm^3) and triethylamine (0.070 cm^3). The solution was cooled to 0 °C, di-*tert*-butyl dicarbonate (1.4 g, 6.4 mmol) in CH_2Cl_2 (3.0 cm^3) was added and the mixture was stirred for 4 d. The evaporation residue, in ethyl acetate, was washed with water and brine. Evaporation and chromatography (ethyl acetate–hexane 1 : 4) gave **3a** (895 mg, 79%) as very pale yellow crystals: mp 188–190 °C (Found: C, 64.20; H, 5.83; N, 5.35. $C_{14}H_{15}N_1O_4$ requires C, 64.36; H, 5.75; N, 5.36%); ν_{max} (KBr)/cm^{-1} 3320, 1720 and 1682; δ_H 1.45 (9 H, s, Bu^t), 6.49 (1 H, d, J = 5.9 Hz, 4-H), 6.50 (1 H, br s, NH), 7.22 (1 H, d, J = 5.9 Hz, 3-H), 7.42 (1 H, t, J = 7.9 Hz, 7-H), 7.97 (1 H, d, J = 7.9 Hz, 6-H) and 8.02 (1 H, d, J = 7.9 Hz, 8-H); *m/z* 262.1085 (M + H) ($C_{14}H_{16}N_1O_4$ requires 262.1079), 261.1008 (M) ($C_{14}H_{15}N_1O_4$ requires 261.1001) and 206 (M − Bu^t).

5-[*N*,*N*-Bis(1,1-dimethylethoxycarbonyl)amino]isocoumarin 3b

To 5-aminoisocoumarin[17] **2** (100 mg, 0.62 mmol) in dichloromethane (1.0 cm^3) was added DMF (0.5 cm^3) and triethylamine (0.010 cm^3). The solution was cooled to 0 °C and di-*tert*-butyl dicarbonate (403 mg, 1.84 mmol) in dichloromethane (1.0 cm^3) was added during 10 min. The mixture was stirred for 4 d. The evaporation residue, in ethyl acetate, was washed with water and brine. Evaporation and chromatography (ethyl acetate–hexane 1 : 4) gave **3b** (130 mg, 58%) as white crystals: mp 165–167 °C; ν_{max} (KBr)/cm^{-1} 1720; δ_H 1.38 (18 H, s, 2 × Bu^t), 6.47 (1 H, dd, J = 5.9, 0.6 Hz, 4-H), 7.32 (1 H, d, J = 5.9 Hz, 3-H), 7.53 (1 H, t, J = 7.9 Hz, 7-H), 7.54 (1 H, dd, J = 7.9, 1.3 Hz, 6-H) and 8.29 (1 H, ddd, J = 7.9, 1.3, 0.6 Hz, 8-H); *m/z* 362.1617 (M + H) ($C_{19}H_{24}N_1O_6$ requires 362.1604), 361.1544 (M) ($C_{19}H_{23}N_1O_6$ requires 361.1525), 262 (M + H − Boc) and 206 (M + H − Boc − Bu^t).

5-Iodoisocoumarin 4

Sodium nitrite (2.6 g, 37.3 mmol) in water (200 cm^3) was added to 5-aminoisocoumarin **2**[17] (7.0 g, 43.4 mmol) in aq. hydrochloric acid (4.5 M, 250 cm^3) at 0 °C. A chilled solution of potassium iodide (10.0 g, 60 mmol) in water (250 cm^3) was added during 10 min. The mixture was stirred for 2 h before extraction with ethyl acetate. Evaporation and chromatography (hexane–ethyl acetate 4 : 1) yielded **4** (8.3 g, 70%) as off-white crystals: mp 155–156 °C; δ_H ($(CD_3)_2SO$) 6.75 (1 H, d, J = 5.9 Hz, 3-H), 7.37 (1 H, d, J = 7.9 Hz, 7-H), 7.75 (1 H, d, J = 5.9 Hz, 4-H), 8.22 (1 H, d, J = 7.6 Hz, 6-H) and 8.31 (1 H, d, J = 7.9 Hz, 8-H). This material was used without further purification or

characterisation.

5-Bromo-2-(4-methoxyphenylmethyl)isoquinoline-1(2*H*)-one 6c

5-Bromoisocoumarin **14**[17] (100 mg, 0.44 mmol) in 2-methoxyethanol (1.0 cm^3) was boiled under reflux with 4-methoxybenzylamine (61 mg, 0.44 mmol) for 24 h. Evaporation, chromatography (hexane–ethyl acetate 5 : 1) and trituration (diethyl ether) gave **6c** (40 mg, 27%) as white crystals: mp 97–100 °C (lit.[11] mp 98–100 °C); ν_{max} (KBr)/cm^{-1} 1640, 1610 and 690; δ_H 3.79 (3 H, s, Me), 5.15 (2 H, s, CH_2), 6.82 (1 H, dd, J = 7.6, 0.5 Hz, 4-H), 6.87 (2 H, d, J = 8.5 Hz, Ph 3,5-H_2), 7.18 (1 H, d, J = 7.6 Hz, 3-H), 7.27 (2 H, J = 8.5 Hz, Ph 2,6-H_2), 7.33 (1 H, t, J = 8.0 Hz, 7-H), 7.87 (1 H, dd, J = 8.0, 1.5 Hz, 6-H) and 8.43 (1 H, ddd, J = 8.0, 1.5, 0.5 Hz, 8-H).

1-Phenylmethoxyisoquinoline 7

Isoquinolin-1(2*H*)-one **1a** was treated with triphenylphosphine, DEAD and **5d**, as for the synthesis of **10a**, except that the chromatographic eluant was ethyl acetate, to give **7** (39%) as a colourless oil: δ_H 5.58 (2 H, s, CH_2), 7.25 (1 H, d, J = 6.2 Hz, 4-H), 7.36 (5 H, m, Ph-H_5), 7.55 (1 H, dd, J = 8.5, 8.2 Hz, 7-H), 7.66 (1 H, t, J = 8.2 Hz, 6-H), 7.70 (1 H, d, J = 8.2 Hz, 5-H), 8.00 (1 H, d, J = 6.2 Hz, 3-H) and 8.31 (1 H, d, J = 8.5 Hz, 8-H); MS (EI^+) *m/z* 235.0997 (M) ($C_{16}H_{13}ON$ requires 235.0989).

2-(2-Thienylmethyl)isoquinolin-1(2*H*)-one 9c

2-Chloromethylthiophene[23] **8c** (270 mg, 2.0 mmol) and sodium iodide (5 mg) were added to isoquinolin-1-one **1a** (200 mg, 1.4 mmol) and lithium bis(trimethylsilyl)amide (1.0 M in THF, 2.8 mL, 2.8 mmol) in dry DMF (10 cm^3) and the mixture was stirred for 2 d under Ar. The evaporation residue, in ethyl acetate, was washed with water (2×) and brine (2×) and was dried. Evaporation and chromatography (ethyl acetate–hexane 1 : 1) gave **9c** (150 mg, 45%) as a pale buff glass; ν_{max} (KBr)/cm^{-1} 1640; δ_H 5.34 (2 H, s, CH_2), 6.49 (1 H, d, J = 7.3 Hz, isoquinoline 4-H), 6.95 (1 H, dd, J = 5.1, 3.5 Hz, thiophene 4-H), 7.11 (1 H, br d, J = 3.5 Hz, thiophene 3-H), 7.14 (1 H, d, J = 7.3 Hz, isoquinoline 5-H), 7.24 (1 H, dd, J = 5.3, 1.4 Hz, thiophene 5-H), 7.5 (2 H, m, isoquinoline 3,7-H_2), 7.62 (1 H, t, J = 7.5 Hz, isoquinoline 6-H) and 8.45 (1 H, d, J = 7.5 Hz, isoquinoline 8-H); δ_C 46.5, 106.6, 125.9, 126.1, 126.8, 126.9, 127.3, 128.0 (2 × C), 130.6, 132.3, 136.9, 138.8 and 161.9; *m/z* 242.0635 (M + H) ($C_{14}H_{11}NOS$ requires 242.0639).

1-(5-Nitro-2-thienylmethoxy)isoquinoline 10a

Isoquinolin-1(2*H*)-one **1a** (90 mg, 0.62 mmol) was stirred with triphenylphosphine (330 mg, 1.3 mmol) in dry tetrahydrofuran (20 cm^3) under Ar for 5 min. DEAD (209 mg, 1.2 mmol) was added dropwise. After 15 min, (5-nitro-2-thienyl)methanol[18] **8d** (100 mg, 0.63 mmol) was added and the mixture was stirred for 16 h. Evaporation and chromatography (ethyl acetate–hexane 1 : 1) gave **10a** (67 mg, 38%) as a pale buff oil: ν_{max} (film)/cm^{-1} 1631; δ_H 5.74 (2 H, s, CH_2), 7.12 (1 H, d, J = 4.0 Hz, thiophene 3-H), 7.30 (1 H, d, J = 6.0 Hz, isoquinoline 4-H), 7.55 (1 H, dd, J = 8.2, 7.7 Hz, isoquinoline 7-H), 7.70 (1 H, t, J = 7.7 Hz, isoquinoline 6-H), 7.75 (1 H, d, J = 7.7 Hz, isoquinoline 5-H), 7.80 (1 H, d, J = 4.0 Hz, thiophene 4-H), 8.00 (1 H, d, J = 6.0 Hz, isoquinoline 3-H) and 8.25 (1 H, d, J = 8.2 Hz, isoquinoline 8-H); δ_C 62.3, 116.1, 126.1, 127.4, 128.1, 128.5, 130.0, 132.3, 131.8, 131.5, 148.2, 158.9 and 165.6; *m/z* 287.0486 (M + H) ($C_{14}H_{10}N_2O_3S$ requires 287.0490). [1]

5-Iodo-1-(5-nitro-2-thienylmethoxy)isoquinoline 10b

5-Iodoisoquinolin-1(2*H*)-one **1b** was treated with triphenylphosphine, DEAD and **8d**, as for the synthesis of **10a**, to give **10b** (30%) as a yellow powder: mp 78–82 °C (Found: C, 40.7; H, 2.09; N, 6.4. $C_{14}H_9IN_2O_3S$ requires C, 40.87; H, 2.18; N, 6.80%); ν_{max} (KBr)/cm^{-1} 1618; δ_H 5.74 (2 H, s, CH_2), 7.12 (1 H, d, J = 3.9 Hz, thiophene 3-H), 7.27 (1 H, dd, J = 7.4, 8.5 Hz, isoquinoline 7-H), 7.49 (1 H, d, J = 6.3 Hz, isoquinoline 4-H), 7.83 (1 H, d, J = 3.9 Hz, thiophene 4-H), 8.10 (1 H, d, J = 6.3 Hz, isoquinoline 3-H), 8.22 (1 H, d, J = 7.4 Hz, isoquinoline 6-H) and 8.27 (1 H, d, J = 8.5 Hz, isoquinoline 8-H); δ_C 62.8, 96.9, 119.7, 120.0, 124.3, 125.9, 127.9, 139.6, 140.5, 141.7, 147.6 and 158.9; MS (EI^+) *m/z* 412 (M).

5-Bromo-1-(5-nitro-2-thienylmethoxy)isoquinoline 10c

5-Bromoisoquinolin-1(2*H*)-one[2] **1c** was treated with triphenylphosphine, DEAD and **8d**, as for the synthesis of **10a**, to give **10c** (41%) as a yellow powder: mp 128–130 °C; ν_{max} (KBr)/cm^{-1} 1616; δ_H 5.75 (2 H, s, CH_2), 7.13 (1 H, d, J = 4.2 Hz, thiophene 3-H), 7.42 (1 H, dd, J = 8.2, 7.8 Hz, isoquinoline 7-H), 7.65 (1 H, d, J = 6.0 Hz, isoquinoline 4-H), 7.84 (1 H, d, J = 4.2 Hz, thiophene 4-H), 7.97 (1 H, d, J = 7.8 Hz, isoquinoline 6-H), 8.12 (1 H, d, J = 6.0 Hz, isoquinoline 3-H) and 8.25 (1 H, d, J = 8.2 Hz, isoquinoline 8-H); δ_C 62.9, 115.2, 120.1, 121.5, 123.7, 126.1, 127.5, 128.2, 134.7, 139.0, 140.6, 147.8 and 158.9; *m/z* 366.9575 (M + H) ($C_{14}H_9{}^{81}BrN_2O_3S$ requires 366.9589).

1,2-Dimethyl-3-(5-iodo-1-oxo-2*H*-isoquinolin-2-ylmethyl)-5-methoxy-1*H*-indole-4,7-dione 12b

5-Iodoisoquinolin-1(2*H*)-one **1b** was treated with triphenylphosphine, DEAD and **11b**, as for the synthesis of **10a**, to give **12b** (36%) as a purple powder: mp >230 °C; ν_{max} (KBr)/cm^{-1} 1702; δ_H 2.47 (3 H, s, indole 2-Me), 3.81 (3 H, s, NMe), 3.88 (3 H, s, OMe), 5.29 (2 H, s, CH_2), 5.62 (1 H, s, indole 6-H), 6.66 (1 H, d, J = 7.9 Hz, isoquinoline 4-H), 7.12 (1 H, t, J = 7.8 Hz, isoquinoline 7-H), 7.80 (1 H, d, J = 7.9 Hz, isoquinoline 3-H), 8.11 (1 H, dd, J = 7.8, 1.1 Hz, isoquinoline 6-H) and 8.38 (1 H, brd, J = 8.2 Hz, isoquinoline 8-H); δ_C 10.2, 32.6, 42.2, 56.5, 106.5, 109.1, 121.1, 126.7, 127.2, 128.0, 128.7, 134.1, 138.7, 142.5, 138.9, 159.0, 161.1 and 178.1; *m/z* 489.0309 (M + H) ($C_{21}H_{18}IN_2O_4$ requires 489.0311). [2]

3-(5-Bromo-1-oxo-2*H*-isoquinolin-2-ylmethyl)-1,2-dimethyl-5-methoxy-1*H*-indole-4,7-dione 12c

5-Bromoisoquinolin-1(2*H*)-one **1c** was treated with triphenylphosphine, DEAD and **11b**, as for the synthesis of **10a**, to give **12c** (36%) as a purple powder: mp 278–280 °C; ν_{max} (KBr)/cm^{-1} 1695; δ_H 2.47 (3 H, s, indole 2-Me), 3.81 (3 H, s, NMe), 3.88 (3 H, s, OMe), 5.30 (2 H, s, CH_2), 5.62 (1 H, s, indole 6-H), 6.77 (1 H, d, J = 8.0 Hz, isoquinoline 4-H), 7.27 (1 H, dd, J = 7.8, 7.3 Hz, isoquinoline 7-H), 7.82 (1 H, d, J = 8.0 Hz, isoquinoline 3-H), 7.84 (1 H, dd, J = 7.3, 0.9 Hz, isoquinoline 6-H) and 8.36 (1 H, br d, J = 7.8 Hz, isoquinoline 8-H); δ_C 10.2, 32.6, 42.2, 56.5, 104.3, 106.6, 116.1, 120.4, 121.2, 126.8, 127.4, 128.1, 128.5, 128.8, 134.1, 135.6, 136.3, 138.8, 159.3, 161.2 and 178.2; *m/z* 441.0443 (M + H) ($C_{21}H_{18}N_2O_4Br$ requires 441.0449).

1,2-Dimethyl-3-[5-(1,1-dimethylethoxycarbonylamino)-1-oxo-2*H*-isoquinolin-2-ylmethyl]-5-methoxy-1*H*-indole-4,7-dione 12d

Compound **1d** was treated with triphenylphosphine, DEAD and **11b**, as for the synthesis of **10a**, to give **12d** (12%) as an orange solid: mp >230 °C; δ_H 1.54 (9 H, s, Bu^t), 2.47 (3 H, s, indole 2-Me), 3.81 (3 H, s, NMe), 3.88 (3 H, s, OMe), 5.25 (2 H, s, CH_2), 5.62 (1 H, s, indole 6-H), 6.57 (1 H, d, J = 8.0 Hz, isoquinoline 4-H), 7.27 (1 H, t, J = 8.0 Hz, isoquinoline 7-H), 7.47 (2 H, m, isoquinoline 3,6-H_2) and 8.21 (1 H, d, J = 7.8 Hz, isoquinoline 8-H).

1,2-Dimethyl-3-(isoquinolin-1-yloxymethyl)-1*H*-indole-4,7-dione 13a

Isoquinolin-1(2*H*)-one **1a** was treated with triphenylphosphine, DEAD and **11b**, as for the synthesis of **10a**, to give **13a** (40%) as

a red powder: mp >230 °C; ν_{max} (KBr)/cm^{-1} 1725, 1694; δ_H 2.38 (3 H, s, indole 2-Me), 3.80 (3 H, s, NMe), 3.89 (3 H, s, OMe), 5.62 (1 H, s, indole 6-H), 5.72 (2 H, s, CH_2), 7.20 (1 H, d, J = 5.8 Hz, isoquinoline 4-H), 7.45 (1 H, td, J = 8.2, 1.1 Hz, isoquinoline 7-H), 7.61 (1 H, td, J = 8.2, 1.1 Hz, isoquinoline 6-H), 7.70 (1 H, d, J = 8.2 Hz, isoquinoline 5-H), 8.00 (1 H, d, J = 5.8 Hz, isoquinoline 3-H) and 8.17 (1 H, d, J = 8.6 Hz, isoquinoline 8-H); δ_C 9.8, 50.8, 53.4, 56.4, 114.8, 117.3, 119.8, 122.0, 124.4, 125.9, 126.4, 129.0, 130.3, 137.9, 138.0, 139.6, 142.4, 159.7, 160.3, 177.6 and 178.9; MS (EI$^+$) *m/z* 363.1346 (M) ($C_{21}H_{18}N_2O_4$ requires 363.1344).

1,2-Dimethyl-3-(5-iodoisoquinolin-1-yloxymethyl)-5-methoxy-1*H*-indole-4,7-dione 13b

2-Iodoisoquinolin-1(2*H*)-one **1c** was treated with triphenylphosphine, DEAD and **11b**, as for the synthesis of **10a**, to give **13b** (39%) as a purple powder: mp >230 °C; ν_{max} (KBr)/cm^{-1} 1702; δ_H 2.37 (3 H, s, indole 2-Me), 3.81 (3 H, s, NMe), 3.88 (3 H, s, OMe), 5.62 (1 H, s, indole 6-H), 5.71 (2 H, s, CH_2), 7.16 (1 H, dd, J = 8.3, 7.4 Hz, isoquinoline 7-H), 7.40 (1 H, d, J = 6.1 Hz, isoquinoline 4-H), 8.08 (1 H, d, J = 6.1 Hz, isoquinoline 3-H), 8.15 (1 H, dd, J = 7.4, 1.0 Hz, isoquinoline 6-H) and 8.20 (1 H, br d, J = 8.3 Hz, isoquinoline 8-H); δ_C 9.9, 32.4, 56.4, 58.6, 106.6, 117.0, 118.5, 120.6, 125.1, 127.5, 128.9, 134.5, 138.0, 139.5, 141.1, 159.7, 160.5, 177.6 and 178.9; *m/z* 489.0309 (M + H) ($C_{21}H_{18}IN_2O_4$ requires 489.0311).

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